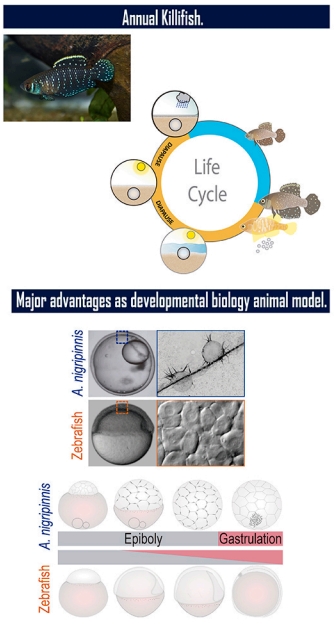
N-Cadherin affects mitotic index and epithelial cell shape during early morphogenesis in killifish embryos.

Sánchez, W. (1,2); Concha. M.L. (2,3); **Reig,G.** (4)

(1) Single Molecule Biochemistry and Mechanobiology laboratory, Universidad de Chile, Santiago, Chile; (2) Biomedical NeuroscienceInstitute, Santiago, Chile; (3) Center for Geroscience, Brain Health and Metabolism, Santiago, Chile; (4) Escuela de Tecnología MédicaCentro Integrativo de Biología y Química Aplicada, Universidad Bernardo O'Higgins, Santiago, Chile.

Abstract

We are interested in how shape emerges in the developing embryo focusing in the dynamic functionality associated with Cadherins molecules. In this study we analyze the expression and functionality of classical N-Cadherin along the embryonic-extraembryonic cell interface during early morphogenesis in killifish embryo. We reveal that N-cadherin transcripts are maternally provided and protein express dynamically covering embryonic deep cells layer (DCL) as well as extra-embryonic structures such as epithelial enveloping cell layer (EVL) and yolk syncytial layer (YSL). Loss of function analysis demonstrate that mitotic cell division is affected. Using time-lapse confocal microscopy we noticed that the splitting of genomic DNA during mitosis is compromised in DN-Ncad derived embryos. Although multinucleate EVL cells are a common feature in this fish specie, EVL derived from DN-Ncad embryos are bigger in size and showed differences in topological distribution of polygonal cell shapes along the epithelia. Thus, N-cadherin seems to play a role during mitosis and the cellular and molecular signaling involved in this functionality will be addressed soon. Furthermore, as E- and N-Cadherin are coexpressed within same cell types understanding the functional crosstalk between will be addressed soon. Furthermore, as E- and N-Cadherin are co-expressed within same cell types understanding the functional crosstalk between will be a necessary step to better understand the complexities of morphogenesis.



1.N-Cadherin is maternally provided and becomes progresivelly enriched within DCL and YSL.

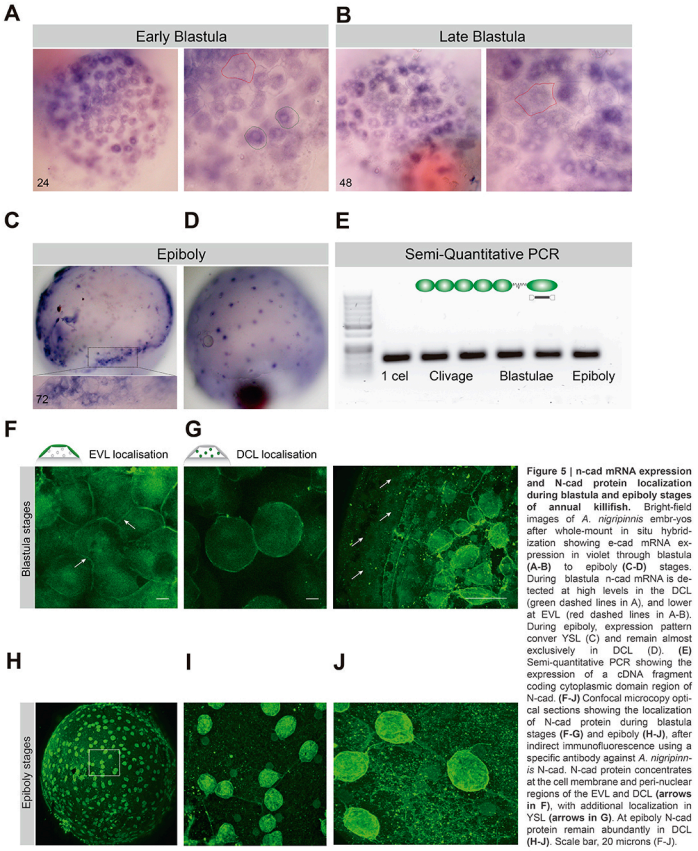


Figure 5 | n-cad mRNA expression and N-cad protein localization during blastula and epiboly stages of annual killifish. Bright-field images of *A. nigripinnis* embryos after whole-mount in situ hybridization showing e-cad mRNA expression in violet through blastula **(A-B)** to epiboly **(C-D)** stages. During blastula n-cad mRNA is detected at high levels in the DCL (green dashed lines in A), and lower at EVL (red dashed lines in A-B). During epiboly, expression pattern conver YSL (C) and remain almost exclusively in DCL (D). **(E)** Semi-quantitative PCR showing the expression of a cDNA fragment coding cytoplasmic domain region of N-cad. **(F-J)** Confocal microcopy optical sections showing the localization of N-cad protein during blastula stages **(F-G)** and epiboly **(H-J)**, after indirect immunofluorescence using a specific antibody against *A. nigripinnis* N-cad. N-cad protein concentrates at the cell membrane and peri-nuclear regions of the EVL and DCL **(arrows in F)**, with additional localization in YSL **(arrows in G)**. At epiboly N-cad protein remain abundantly in DCL **(H-J)**. Scale bar, 20 microns (F-J).

2.Splitting of genomic DNA during mitosis is compromised in DN-Ncad derived embryos.

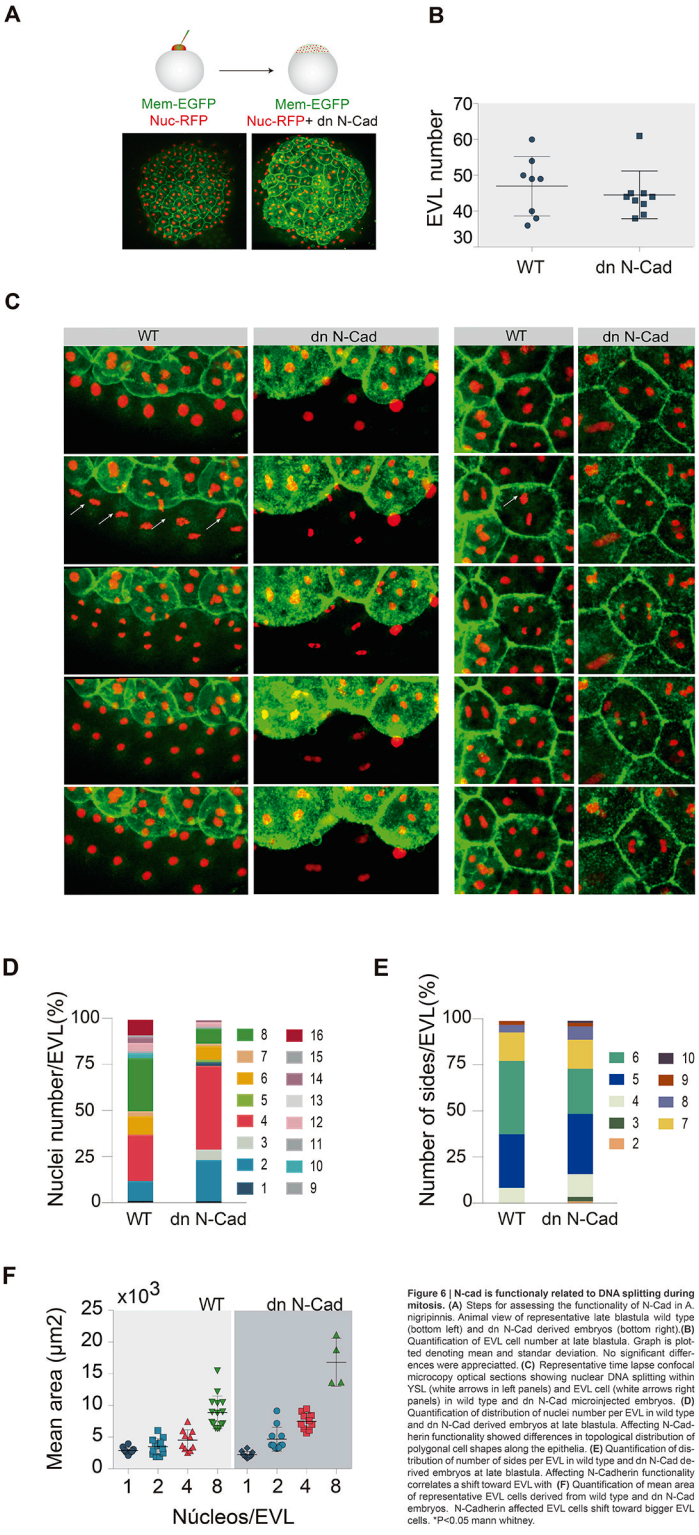


Figure 6 | N-cad is functionaly related to DNA splitting during mitosis. (A) Steps for assessing the functionality of N-Cad in A. nigripinnis. Animal view of representative late blastula wild type (bottom left) and dn N-Cad derived embryos (bottom right).**(B)** Quantification of EVL cell number at late blastula. Graph is plotted denoting mean and standar deviation. No significant differences were appreciatted. **(C)** Representative time lapse confocal microcopy optical sections showing nuclear DNA splitting within YSL (white arrows in left panels) and EVL cell (white arrows right panels) in wild type and dn N-Cad microinjected embryos. **(D)** Quantification of distribution of nuclei number per EVL in wild type and dn N-Cad derived embryos at late blastula. Affecting N-Cadherin functionality showed differences in topological distribution of polygonal cell shapes along the epithelia. **(E)** Quantification of distribution of number of sides per EVL in wild type and dn N-Cad derived embryos at late blastula. Affecting N-Cadherin functionality correlates a shift toward EVL with **(F)** Quantification of mean area of representative EVL cells derived from wild type and dn N-Cad embryos. N-Cadherin affected EVL cells shift toward bigger EVL cells. *P<0.05 mann whitney.