# REGULATION OF ZYXIN WITH ALTERED HEMODYNAMICS IN DEVELOPING CHICK HEART

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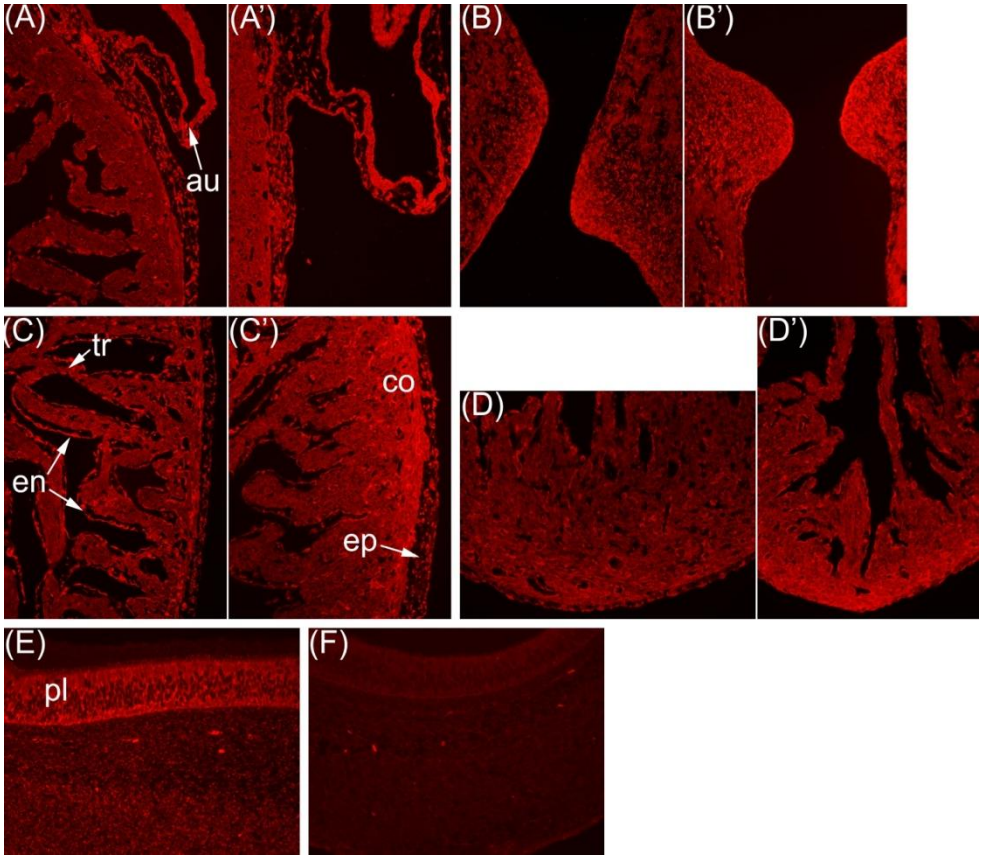
Zyxin, an actin-associated protein, involves in cytoskeletal response to mechanical stress by localizing to sites of increased loading. In addition, zyxin is indicated as a factor in cell migration by impacting cell-cell adhesion. It is implicated that zyxin also plays a role in cardiac development. The developing heart begins as a single tube and develops into a complex four-chambered heart. Mechanical forces imposed by blood flow and pressure on the walls of the cardiac chambers and vessels influence heart development. Various experimental manipulations of blood volume, course, or rate of intracardiac blood flow produce cardiovascular anomalies and alter cardiomyocyte assembly. Our study aims to answer questions concerning the roles of zyxin in early cardiac development. We hypothesized that zyxin expression is prevalent in heart tissue with increased mechanical stress during the differentiation of cardiomyocytes.

We used chick embryo to provide a system that can be manipulated *in ovo*, and avoid organismal death or mother-fetus confounding factors. A small window was made from the blunt-end of the egg to expose the embryo at stage 21 (3½-d) The outflow tract of the heart was partially constricted by tying a loop of 10-0 nylon around the midpoint of the conotruncus. The window was then sealed with parafilm, and the egg was returned to the incubator until stage-34 (8-d). Normal embryos were left unaltered. Western blot analyses were performed from the homogenate of atrial or ventricular tissue with chick B71 anti-zyxin antibody to label the zyxin in cardiac tissue. Zyxin localization of the atrium or ventricle was identified through immunohistochemical (IHC) staining with chromogenic Vectastain ABC, and immunofluorescence (IF) staining with Alexa fluor 594. Fluorescent intensity of the zyxin expression from various regions of the normal versus conotruncal-banded hearts were analyzed using Image J software. Data ($n \geq 20$) are presented as mean±SEM, and analyzed by Student's *t*-test with statistical significance defined as a *p* value of less than 5% ($p<0.05$).

Western blot assay indicated a fair amount of zyxin expressed in both atrial and ventricular tissues. Both IHC and IF staining of banded hearts displayed an increase in zyxin expression in both atrial and ventricular epi- and endocardium, but most prominently in the atrioventricular cushion and ventricular apex with increased mechanical load. Zyxin localization analysis of the IF images via Image J confirmed a significant increase in areas of fluorescence intensity in the conotruncal-banded hearts ($p<0.05$), particularly the atrioventricular cushion and compact layer of the left ventricle, as well as the ventricular apex.

Considering zyxin's role in cellular migration and cytoskeletal response to increased loads, it is strongly implicated that zyxin plays an important role in early heart development. Since a number of congenital heart diseases are associated particularly with atrioventricular canal development, zyxin likely plays

a significant role in this process. This area is relevant to the sites of high rates of cellular migration (and cell death) where increased blood force and pressure against the walls of the conduit. The increased blood flow also alters the endothelial alignment of the atrioventricular cushion, potentially due to change in surface shear stress. The development of the atrioventricular canal is crucial for mature valve formation with alterations in the process would lead to subsequent malformed valves.




Results of the immunofluorescence staining with Alexa Flour 594 in both left atrium and along the superior in normal (A) and conotruncal (CT)-banded (A') hearts, respectively. Fluorescence brightness is more prominent in the atrioventricular cushion in the CT-banded (B') when compared to normal (B) heart. Red fluorescence stain is visible in both epi- and endocardium of normal (C) and CT-banded (C') hearts, but brightness intensity extends much towards the inner compact layer (C') as well as the apex of the ventricle (D') in the CT-banded heart. (E) Chick gizzard serves as positive control, and shows distinct fluorescence in the proventriculus longitudinal folds (pl). (F) Pre-immune serum treated section of chick gizzard serves as negative control. (au: *auricle*, en: *endothelium*; ep: *epicardium*; co: *compact layer*; tr: *trabeculae.*)