Intercalated Disc Protein, mXinα, negatively modulates the Branching activity of p120-catenin via its Direct Interaction

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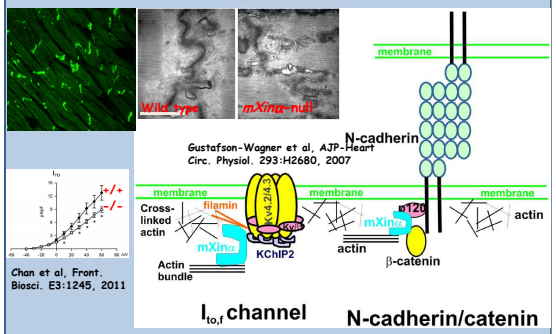
Introduction

Xin repeat-containing family of proteins, Xinα (Xirp1) and Xinβ (Xirp2), localizes to the intercalated discs (ICDs) of mammalian hearts.

Mouse Xinα (mXinα) not only directly interacts with β-catenin but also bundles actin filaments, suggesting mXina plays linkage role between N-cadherin/catenin complex and actin cytoskeleton.

Proposed roles for mXinα at ICD:

1. modulate ICD activity & integrity
2. influence $I_{to,f}$ channel surface expression



Abstract: questions & results

Does mXinα interact with p120-catenin? Yes

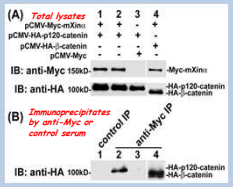
directly or indirectly? Directly

Where is the p120-catenin-binding sites on mXinα? multiple binding sites, different from previously identified β-catenin-binding domain

What are functional consequences of the interaction between mXinα and p120-catenin? negatively modulate the p120-catenin-induced branching phenotype

how? affect Rho GTPases activities?

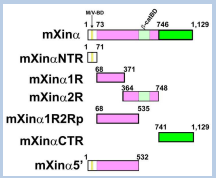
Similar to β-catenin, force expressed p120-catenin in CHO cells is co-pelleted with mXinα (co-IP & pull-down)



(C) *Yeast 2 hybrid with mXinα as bait: identified p120-catenin* (Choi et al, JBC 282:3604, 2007)

(D) *GST bead pull-down*

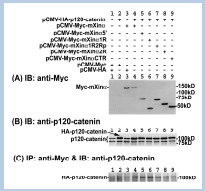
Schematic diagram of mXinα and its fragments



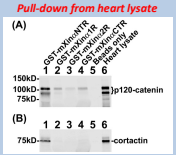
- M/V-BD: Mena/VASP-binding domain
- β-catBD: β-catenin-binding domain

Multiple p120-catenin-binding sites on mXinα

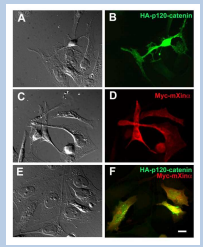
Co-transfection & co-IP

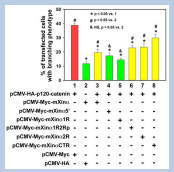


Pull-down from heart lysate

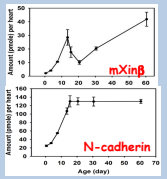


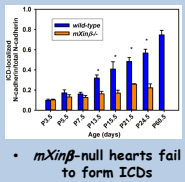
- mXinα NTR, 1R, 2R and CTR fragments were capable of binding & pulling-down p120-catenin, suggesting the existence of multiple binding sites.
- Some of which are different from the known β-catenin binding domain.



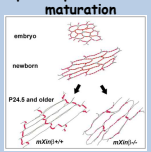


mXinβ initiates the maturation of the intercalated discs

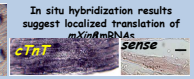




Bi-phasic process of ICD maturation

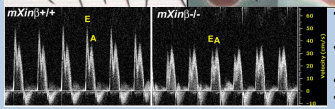


- *mXinβ*-null hearts fail to form ICDs



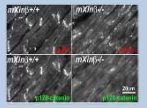
In situ hybridization results suggest localized translation of *mXinβ* mRNAs

mXinβ knockout mice



- Severe growth retardation
- Ventricular Septal Defect
- Diastolic dysfunction
- Early postnatal lethality

Decreased mitral inflow E-wave velocity in mXinβ-null mouse hearts, suggesting a diastolic dysfunction. (Doppler flow spectra)

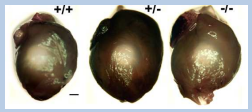


A functional hierarchy between mXinα and mXinβ: Mis-localization of mXinα and p120-catenin in *mXinβ*-null cardiomyocytes

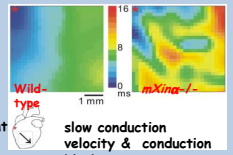
- mXinβ is uniquely up-regulated during the redistribution of N-cadherin from the lateral surface of cardiomyocytes to their termini.

mXinα knockout mice

: Progressive cardiac hypertrophy and cardiomyopathy with conduction defects



- up-regulation of mXinβ in mXinα-deficient hearts
- mXinα directly interacts with β-catenin, p120- catenin, filamin and KChIP2

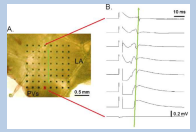


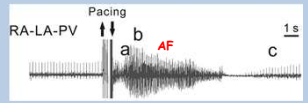
slow conduction velocity & conduction block

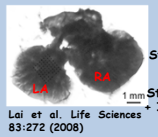
Cardiomyocytes in Pulmonary Veins and atrial fibrillation

- Atrial fibrillation (AF) is the most common adult arrhythmia; increased risk of stroke, heart failure and dementia.
- In AF, rapid and irregular atrial activity overrides normal sino-atrial node function, resulting in irregular impulse conduction to ventricles.(re-entry
- In many cases, ectopic electrical activity originates in the pulmonary veins (PVs) and may serve to trigger and maintain AF.
- *mXinα* deletion prevents the induction of AF

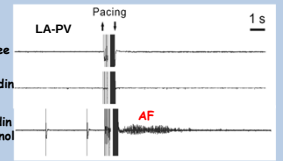
AF induced in wild-type left atrial-pulmonary vein (LA-PV)



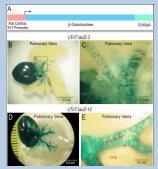




Lai et al. Life Sciences 83:272 (2008)



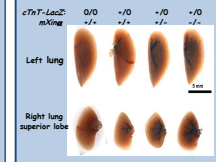
cTnT-LacZ transgenic mice



Kracklauer et al. FEBS J. in press (2012)

- scattered distribution of cardiac cells disconnected from the atrial sleeves
- contained typical sarcomere structures and ICDs
- expressed cardiac isoforms of TnT, TnI and myosin
- contained slightly but clearly distinct cTnT isoform patterns from those in atrial and ventricular muscles.
- their roles in health and diseases (AF)?

cTnT-LacZ; *mXinα* KO mice



mXina-null
: decrease in conduction velocity (LA-PV)
: longer action potential
: prevent the occurrence of reentry paths

More $CT3^+$ (cTnT) cells but no sarcomeric localization (differentiation defect?)

Loss of mXinα results in more cardiomyocytes in PVs. more migration or differentiation?