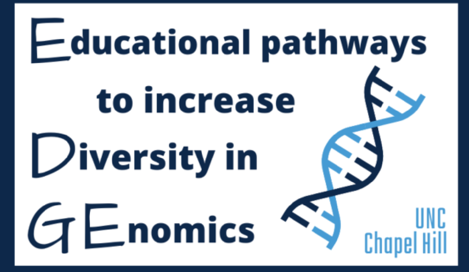
RNA Binding Proteins Regulate Alternative Splicing Networks During Postnatal Atrial Development



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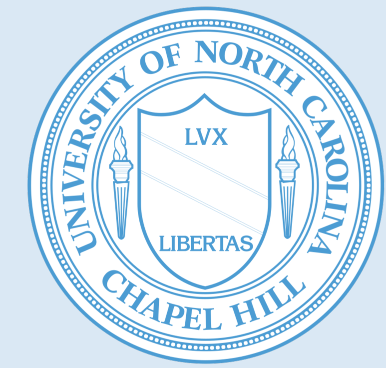
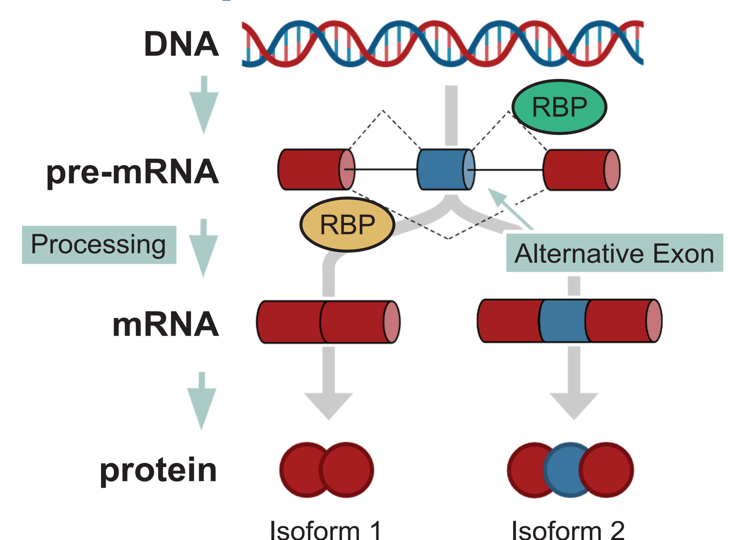


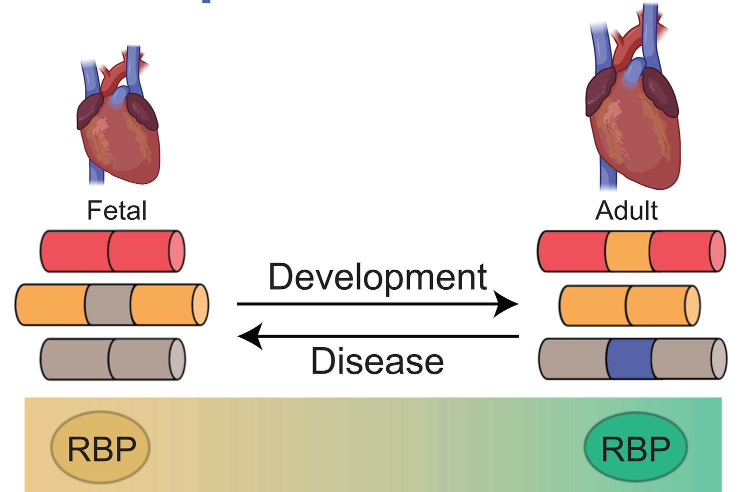
BACKGROUND

Alternative splicing controls isoform production



Alternative splicing increases proteome diversity through the production of multiple protein isoforms from a single gene. RNA-binding proteins (RBPs) influence the inclusion or exclusion of an alternative exon.

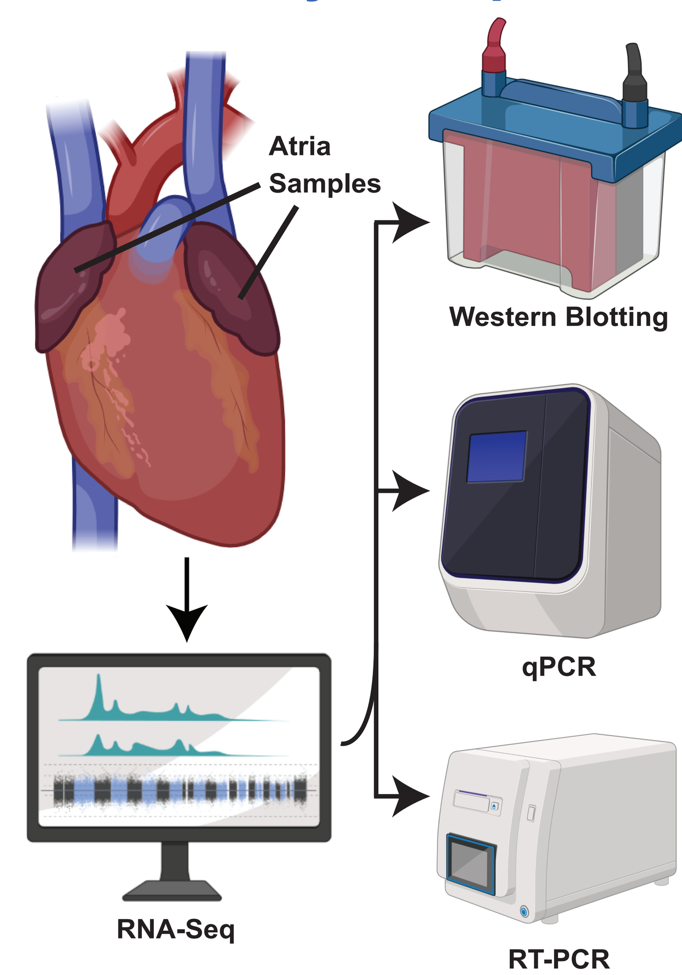
Alternative splicing is regulated during ventricle development and disease



RBPs regulate isoform expression of alternatively spliced genes during heart development[1]. Reversion to abnormal fetal RBP expression in the ventricles can lead to cardiovascular disease[2][3]. **Similarly, we hypothesize that differentially expressed atrial RBPs may be vital for proper postnatal maturation of the atria through the regulation of age-specific splicing networks.**

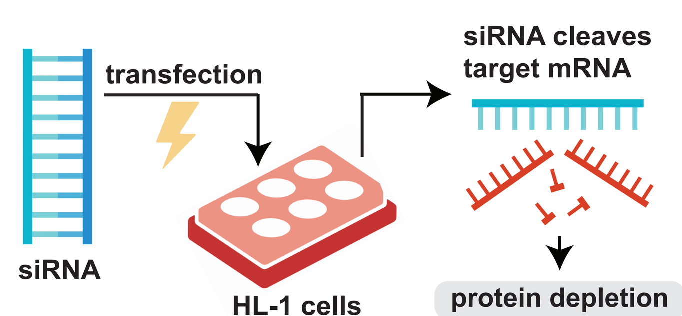
METHODS

RBP Analysis Pipeline



Mouse atria were harvested from 4 time points: post-natal day 4.5 (P4.5), P10, P28, and P90. RNA-seq analysis was performed to identify changes in RBP expression and splicing during development. Western blotting was used to validate protein expression, qPCRs to validate RNA expression, and RT-PCRs to evaluate splicing patterns throughout atria development.

RBP depletion to investigate splicing



Knockdown of Fmr1 was performed in the HL-1 atrial cardiomyocyte cell line to observe splicing patterns and protein expression changes.

RESULTS

Differentially expressed RBPs are candidate regulators of alternative splicing in atria development

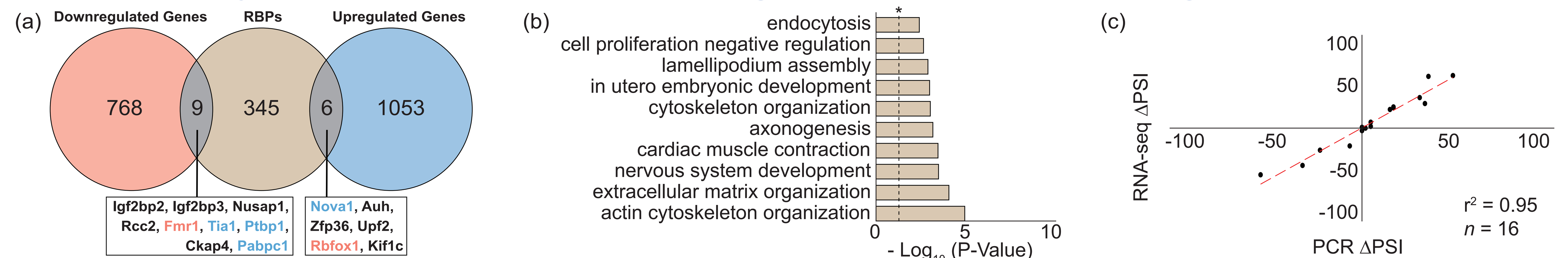


Fig. 1 (a) Differentially expressed genes between P4.5 and P90 were compared to a panel of established RBPs. The differentially established RBPs known to control splicing (shown in blue and orange) were further analyzed by western blotting and qPCR. (b) Gene ontology analysis of alternatively spliced events between P4.5 and P28, revealing a role in atria functions. Filter conditions: count > 3; *P-Value = 0.05 (vertical dashed line). (c) Correlation between RNA-seq and RT-PCR change in percent spliced in (PSI) values of events between P4.5 and P28.

FMR1 is downregulated during atria development

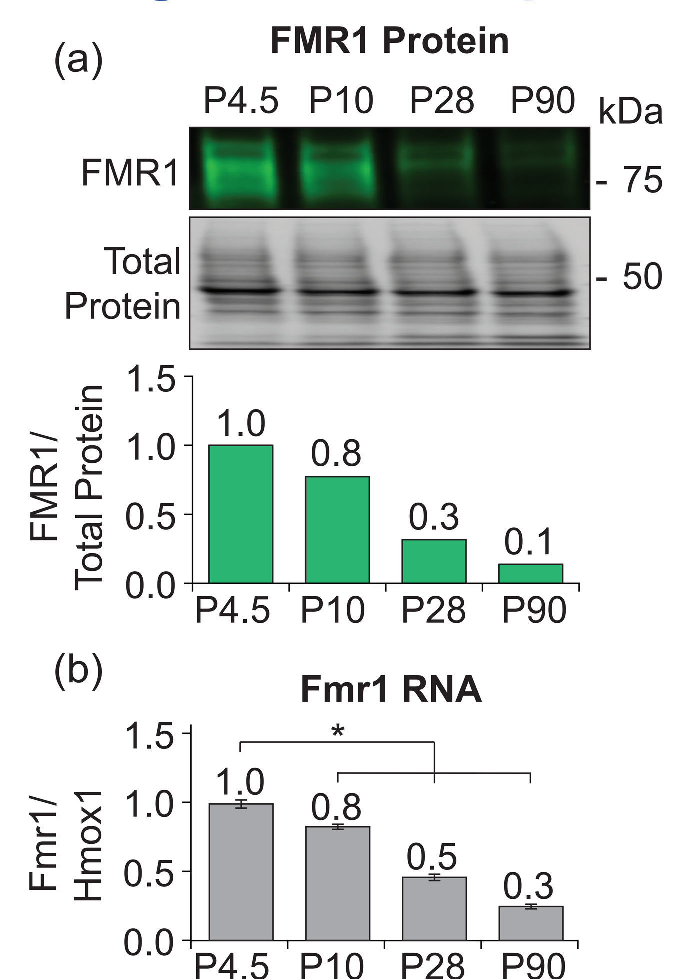


Fig. 2 (a) Western blotting validation of downregulated FMR1 protein expression during atria development. $n = 1$ (b) qPCR validation of Fmr1 mRNA expression, which mirrors protein expression. Quantifications were performed by densitometry. $n = 3$. *P-Value ≤ 0.05 vs P4.5.

RBFOX1 is upregulated during atria development

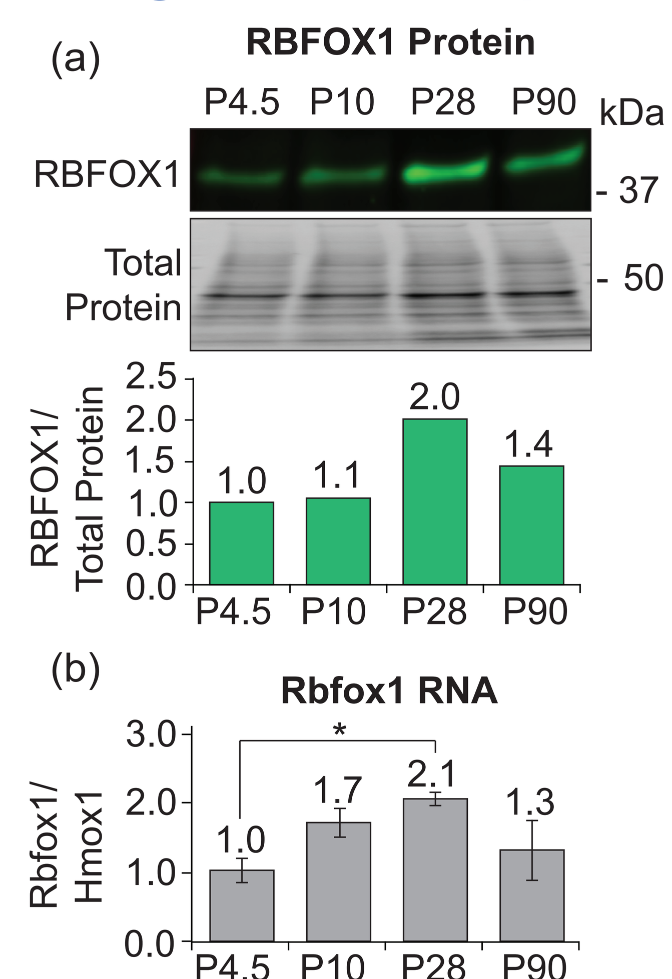


Fig. 3 (a) Western blotting validation of upregulated RBFOX1 protein expression during atria development. $n = 1$ (b) qPCR validation of Rbfox1 mRNA expression, which mirrors protein expression. Quantifications were performed by densitometry. $n = 3$. *P-Value ≤ 0.05 vs P4.5.

Fmr1 splicing changes over heart development

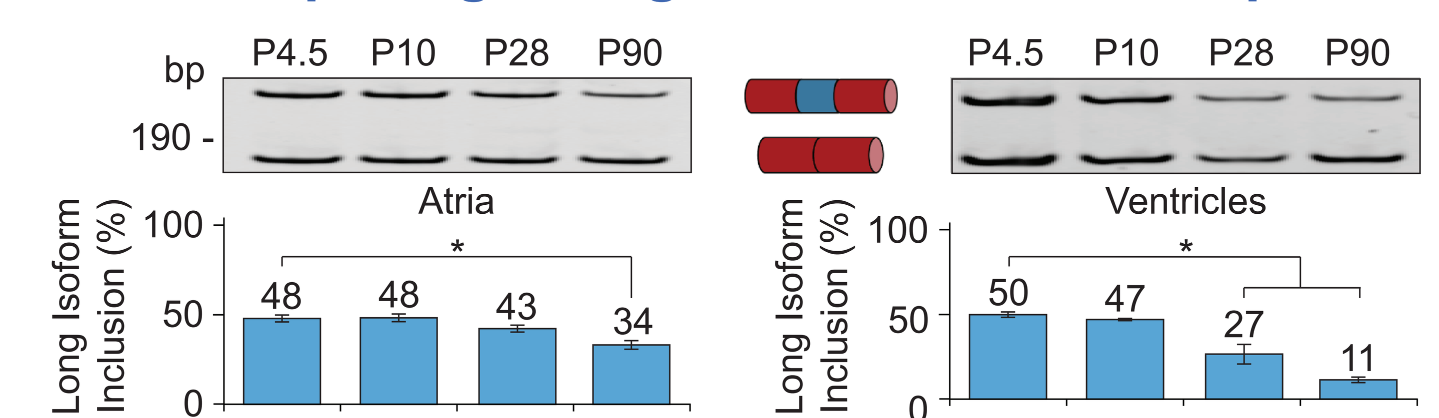


Fig. 4 Splicing of Fmr1 changes in the atria and ventricles, showing lower expression of the long isoform over development, which is more significant in the ventricles. Assayed by RT-PCR. $n = 3$. *P-Value ≤ 0.05 vs P4.5.

Atrial FXR1 expression is unaffected in the absence of FMR1

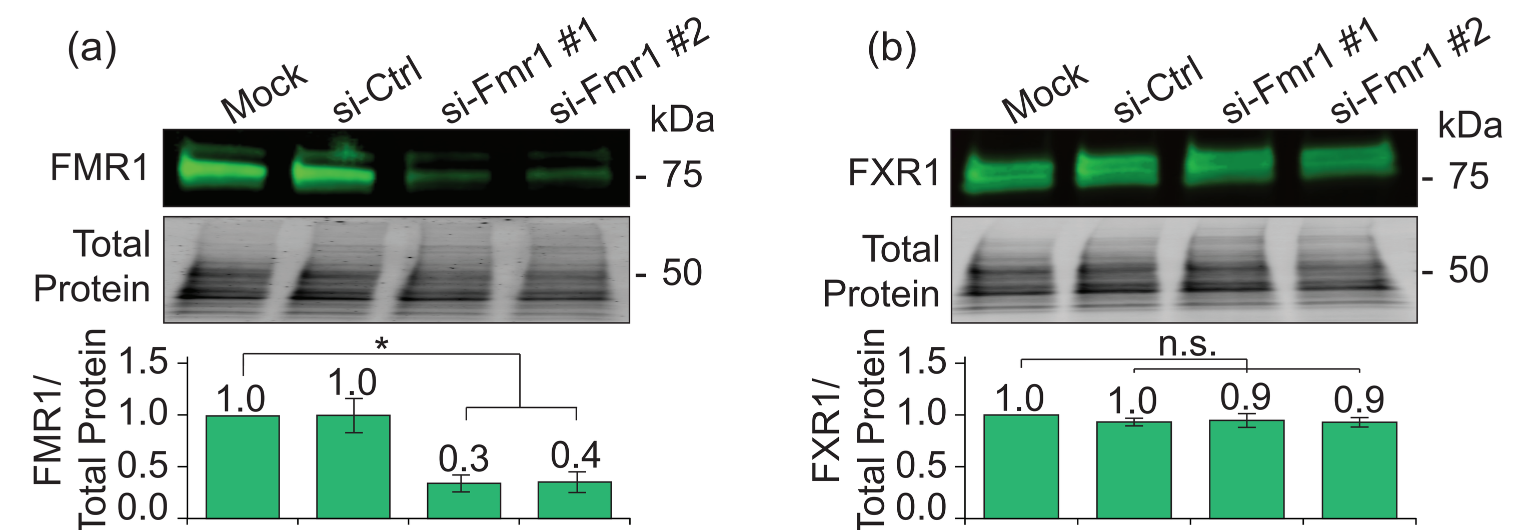


Fig. 5 (a) Western blotting validation of FMR1 depletion in HL-1 cells. $n = 3$. (b) Western blotting to investigate expression of homolog protein FXR1, revealing no protein compensation. Quantifications were performed by densitometry. $n = 3$. *P-Value ≤ 0.05 vs P4.5.

CONCLUSIONS

- Developmentally regulated alternatively spliced genes are enriched in atrial functions
- FMR1 and RBFOX1 are putative regulators of alternative splicing in the atria and may be responsible for proper tissue development
- There is a transition from the long to short Fmr1 splice isoform throughout heart development (more promiment in the ventricles)
- There is no FXR1 protein compensation in the absence of FMR1

NEXT STEPS

- Investigate downstream Fmr1 splicing targets and protein interactions
- Compare RNA and protein expression in the atria with expression in the ventricle and brain
- Similarly to FMR1, explore the splicing targets of RBFOX1 using si-Rbfox1 HL-1 cells
- Observe changes in atria morphology and function within Fmr1^{flox} and Rbfox1^{flox} Cre-lox mouse lines using atrial-specific gene delivery

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CITATIONS

[1] Giudice, J., Xia, Z., Wang, E. *et al.* Alternative splicing regulates vesicular trafficking genes in cardiomyocytes during postnatal heart development. Nat Commun **5**, 3603 (2014).

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