Role of Rho GEFs in regulating the epithelial-mesenchymal transition during gastrulation in chicken embryos

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Abstract

During an epithelial-mesenchymal transition (EMT), epithelial cells lose cell polarity and adhesion, migrate away from the epithelium, and become migratory mesenchymal cells. EMT has numerous downstream functions, such as organ formation, gene expression, and germ layer formation. It is a fundamental evolutionarily conserved process that is executed through multiple mechanisms. EMT is also thought to occur during cancer metastasis. Rho GTPases are known to play a central role in regulating EMT. The activation of Rho GTPases is controlled by Rho GEFs and Rho GAPs. This study investigates Rho GEF function during EMT that occurs during chicken embryo gastrulation. *In situ* hybridization was used to identify Rho GEFs that are expressed during chicken gastrulation and differentially localized around the primitive streak where EMT occurs. Of these, those shown to have an early lethal phenotype when ablated in mice were targeted for further study. Myc-tagged dominant negative and constitutively active forms of these Rho GEFs were created. The constructs were then assayed for their effects on EMT during gastrulation via electroporation into a chicken embryo and immunofluorescence. Several of these constructs affected cell polarity and adhesion. We hypothesize they are activating Rac1, a Rho GTPase. Further investigation will utilize morpholinos to examine the effects of a Rac1 knockout and knockouts of each targeted Rho GEF. This research will lead to an essential understanding of vertebrate gastrulation as well as the underlying mechanisms of cellular metastasis.

Figure 1: Conserved Rho GEF domains that were manipulated to generate dominant negative and constitutively active mutants.

Research question

What is the function of Rho GEFs during the gastrulation EMT of chick embryos?

Hypothesis

Rho GEFs are involved in the regulation of chick EMT polarity. The manipulation of Rho GEFs will cause misregulation of Rho GTPases and thus break EMT.

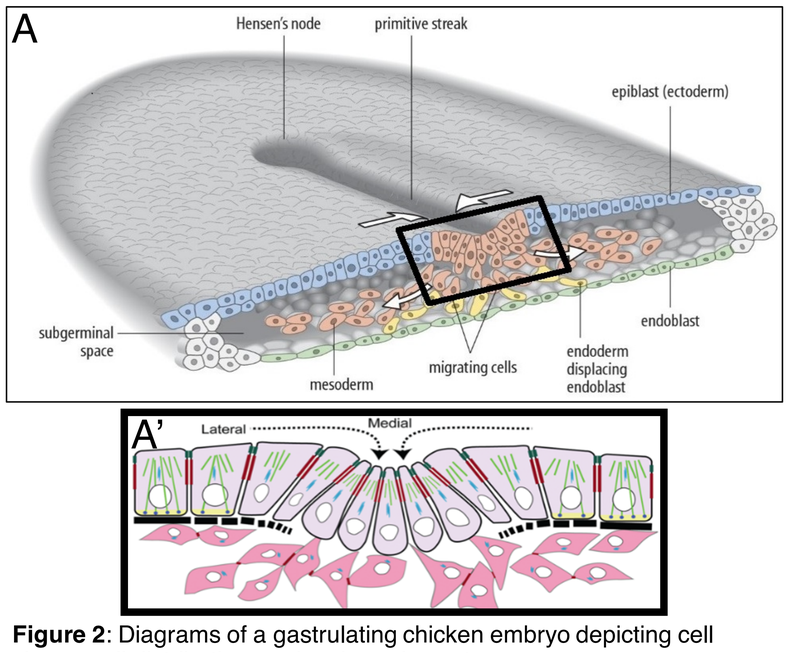
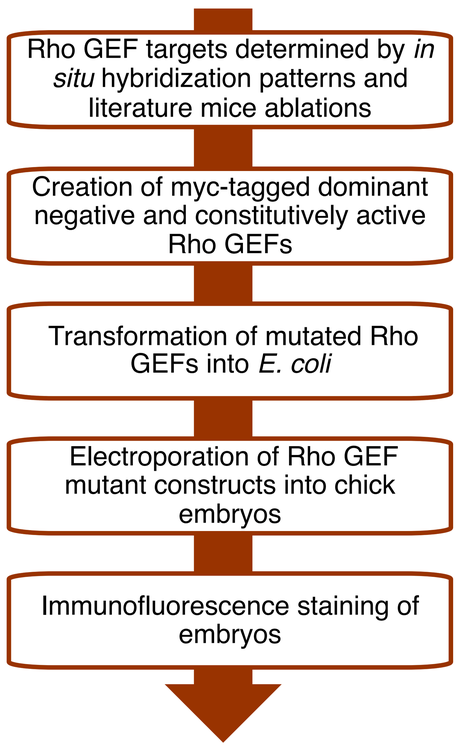


Figure 2: Diagrams of a gastrulating chicken embryo depicting cell shape, cell distribution, and embryonic anatomy.

Methods



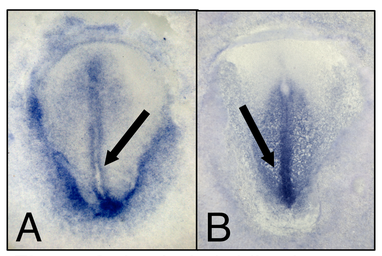


Figure 3: *In situ* hybridization patterns (blue) of two embryos. A is DLG2 and B is PREX1. Arrows denote primitive streak. Images are from a bird's eye perspective (see fig. 2 A).

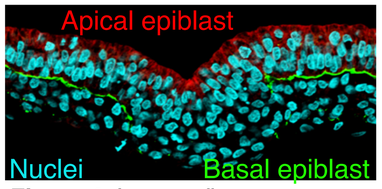


Figure 4: Immunofluorescence staining of an embryo cross section centered at the primitive streak (see fig. 2 A').

Results

- 12 Rho GEFs and their constitutively active and dominant negative constructs tested.
- Three Rho GEFs displayed a phenotype (table 1, fig. 5).

Phenotypic Rho GEF constructs			
Targeted Rho GEF	ECT2	PREX1	TRIO
Apparent phenotype	loss of polarity	loss of polarity	loss of polarity

Table 1: A total of 12 Rho GEFs were targeted. Listed are the ones that displayed a phenotype.

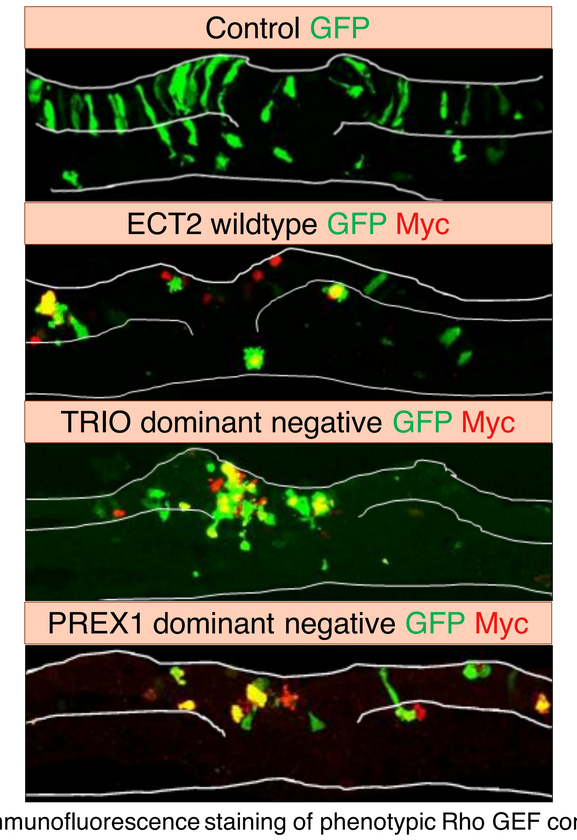


Figure 5: Immunofluorescence staining of phenotypic Rho GEF constructs. All images are of an embryo cross section centered at the primitive streak (see fig. 2 A'). White lines indicate germ layers. Red indicates where the given Rho GEF construct is present. Yellow indicates where both GFP and the Rho GEF construct are present.

Conclusions

- Rho GEFs ECT2, PREX1, and TRIO had phenotypes.
  - ① Loss of polarity
    - Cells lose columnar phenotype and become rounded.
  - ② Lose ability to gastrulate
    - Cells expressing one of these Rho GEFs appear unable to move through the streak and into the mesoderm.
- Previous studies have shown ECT2, PREX1 and TRIO commonly regulate the activation of the Rho GTPase Rac1. Together with our data, this suggests ECT2, PREX1, and TRIO constructs caused a change in Rac1 activation and EMT was misregulated.

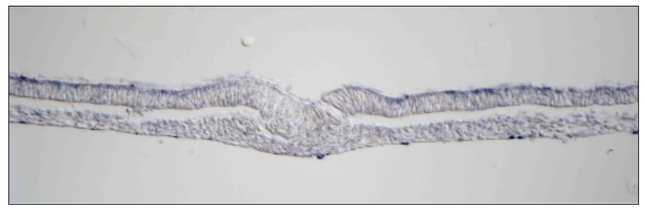


Figure 5: *In situ* hybridization pattern (blue) of Rac1 in an embryo cross section centered at the primitive streak (see fig. 2 A').

New hypothesis

Rac1 is required to maintain epithelial polarity as well as progression of cells through the streak to the mesenchyme during chick gastrulation.

Future directions

- Test remaining Rho GEF mutations.
- Test the effect of Rho GEF morpholinos.
- Assay cell polarity before, during, and after EMT of cells affected by phenotypic Rho GEFs with polarity markers (fig. 6).
- Investigate new hypothesis
  - Create Rac1 mutants (constructs and morpholino) to assay cell polarity and the ability of cells to undergo EMT.
  - Assay cell polarity with polarity markers.

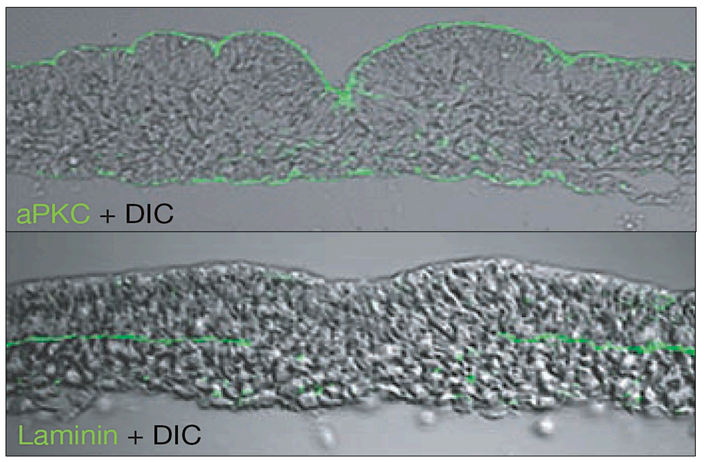


Figure 6: DIC images with immunofluorescently stained polarity makers (aPKC and Laminin). Pictured are embryo cross sections centered at the primitive streak (see fig. 2 A') (Nakaya et al., 2008).

Acknowledgements

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References

Nakaya, Y., Sukowati, W., Wu, Y., and Sheng, G. (2008) RhoA and microtubule dynamics control cell–basement membrane interaction in EMT during gastrulation. Nature Cell Biology 10, 765 – 75.