Reciprocal repression between SOX3 and Snail transcription factors defines embryonic territories at gastrulation.

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RECIPROCAL REPRESSION BETWEEN SOX3 AND SNAIL TRANSCRIPTION FACTORS DEFINES EMBRYONIC TERRITORIES AT GASTRULATION

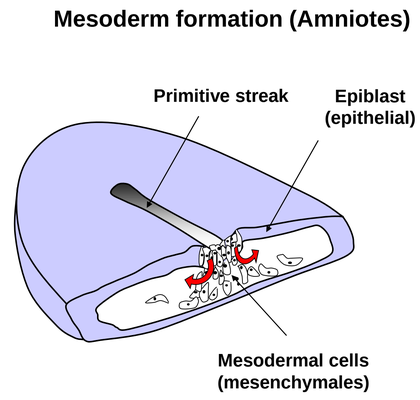
Hervé Acloque[1,3], Oscar Ocaña[1], Ander Mateu[2], Karine Rizzoti[2], Clare Wise[2], Robin Lovell-Badge[2] and M. Angela Nieto[1]

[1]Instituto de Neurociencias de Alicante, CSIC-UMH, 03550 San Joan d'Alacant,Spain, [2]National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK, [3]UMR444 INRA-ENVT Génétique Cellulaire Toulouse, France

INTRODUCTION

With the exception of ectodermal derivatives, all vertebrate tissues are the result of one or several rounds of epithelial-mesenchymal transition (EMT). In the embryo, the first EMT event occurs at gastrulation, when a subset of initial epiblast cells moves to the primitive streak, delaminates and generates the mesoderm and the endoderm. Cells that remain in the epiblast keep their epithelial character and will contribute to the ectodermal derivatives, the epidermis, the ectodermal placodes and the anterior central nervous system (CNS). Indeed, much of the CNS will develop from a subset of the non-ingressing cells later specified as neural precursors. Therefore, it is crucial to identify not only those factors that induce cell ingression at gastrulation but also those that prevent it, as protection from undergoing EMT is necessary to ensure the formation of ectodermal derivatives. Indeed, previous studies have shown that committed neural progenitor cells at the anterior part of the primitive streak are protected from signals that induce internalization but ingression starts at least as early as stage HH2, suggesting that a different mechanism must exist to protect early ectodermal cells from the EMT inducers.

We show that in the chick embryo the decision to internalize is mediated by reciprocal transcriptional repression of Snail2 and Sox3 factors. We also show that the relationship between Sox3 and Snail is conserved in the mouse embryo and in human cancer cells. In the embryo, Snail expressing cells ingress at the primitive streak while Sox3 positive cells, unable to ingress, ensure the formation of ectodermal derivatives. Thus, the subdivision of the early embryo into the two main territories, ectodermal and mesendodermal, is regulated by changes in cell behavior mediated by the antagonistic relationship between Sox3 and Snail transcription factors.



RESULTS

1– *Snail2* overexpression induces ectopic EMT and delamination in the epiblast of the chick blastula

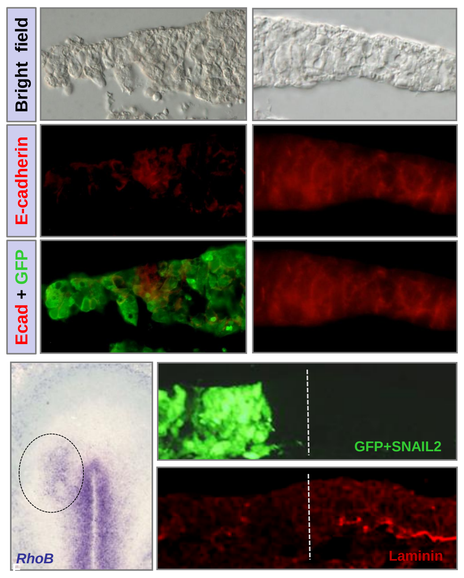
• **Ectopic expression of *Snail2*** (coexpressed with GFP) **alters the epithelial structure** of the epiblast producing a localized ectopic EMT illustrated by the loss of **E-cadherin.**

These morphological changes are accompanied by:

• **Ectopic activation of *RhoB* ,** a small GTPase shown to be activated by Snail2 during neural crest cells delamination and migration (the electroporated area is highlighted by the dotted circle) .

• **Disruption of the basal membrane** determined by the downregulation of laminin expression in the electroporated area. The embryonic midline is showed by a dotted line.

These data indicate that Snail2 is sufficient to trigger EMT and cell delamination from the chick epiblast, suggesting that **the epiblast should be protected from Snail expression in the early embryo.**



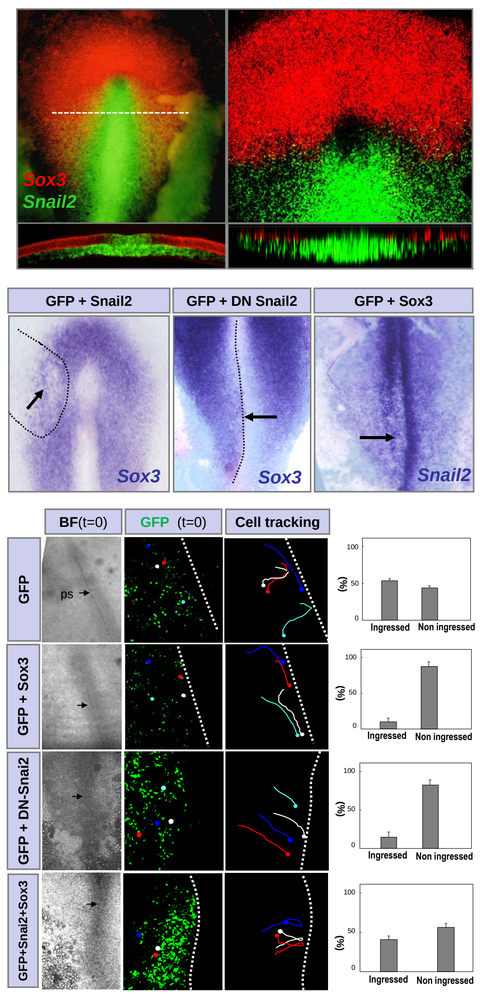
2 – *Sox3* and *Snail2* are complementary expressed and mutually repressed, controlling cell ingression at the primitive streak

• ***Sox3* and *Snail2* are complementary expressed** in the gastrulating embryo. As shown here by fluorescent in situ hybridization in a stage HH3+ chick embryo, *Sox3* is detected in the epiblast delineating the prospective neural plate and is absent from the primitive streak (PS). *Snail2* is expressed in the PS and in the newly formed mesoderm. Confocal analysis confirms that there is no co-expression of *Snail2* and *Sox3.*

• ***Snail2* overexpression in the prospective neural plate represses *Sox3* expression** while overexpression of a dominant negative of *Snail2* (DN-Snail2) extends *Sox3* expression until the embryonic midline. Conversely, **ectopic Sox3 expression represses Snail2** expression in the primitive streak.

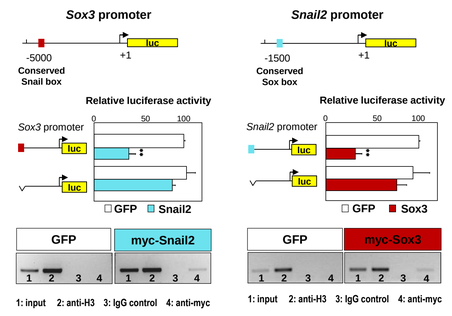
• Time lapse confocal analysis in cultured embryos expressing GFP, GFP plus Sox3, plus DN-Snail2, plus Sox3 and Snail2. **Ectopic Sox3 or DN-Snai2 expression blocks cell ingression at the primitive streak** without affecting the convergence movement towards the midline. Co-expression of exogenous Sox3 and Snail2 at the PS partially rescue the phenotype observed with Sox3 only, confirming that the repression of endogenous Snail2 by Sox3 is the one of the causes that block cell ingression at the streak.

Altogether, these results strongly suggest that the decision to ingress at the primitive streak depends on the interactions between Snail2 and Sox3 transcription factors.



3- Snail2 and Sox3 are direct mutual transcriptional repressors

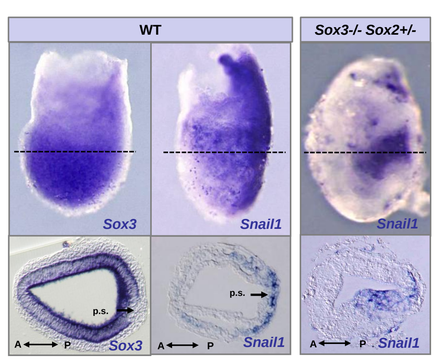
• **Sox3 and Snail2 directly bind and respectively repress Snail2 and Sox3 promoters** through response elements conserved in birds and mammals**.** Snail2 overexpression in chick embryos decreased Sox3 promoter activity (illustrated by a decreased in luciferase activity) while deletion of the conserved response element for Snail abrogate Snail2 effect. Chromatin immunoprecipitation experiments (ChIP) confirm that Snail2 directly binds this response element. Similar experiments were performed overexpressing Sox3 and looking at the Snail2 promoter. Similar results were observed..



4- Snail and Sox3 antagonistic relationship is conserved in mouse embryos

• ***Sox3* and *Snail1* are complementary expressed in the mouse gastrula.** Snail1 is the family member expressed in the primitive streak and early mesendodermal cells in the mouse. Note the similarities in the expression patterns between chick and mouse embryos. Ectopic *Snail1* expression can be observed in the mutant *Sox3*-/-; *Sox2*+/- embryos leading to ectopic EMT in the epiblast. The dotted lines indicate the level of the sections shown in the lower panels.

• **Snail1 is activated in Sox3 deficient embryoid bodies**. EBs (5 days of differentiation) derived from WT mouse ES cells are round and smooth, whereas those derived from *Sox3* null ES cells show irregular edges with budding cells delaminating from the EBs, leading to abundant isolated cells in the culture medium. E-cadherin is strongly downregulated in Sox3-/- compared to wild type (WT) EBs. Conversely, Snail1 mRNA and protein are detected only in *Sox3*-/- EBs, mainly in cells at the edges. These data suggest that the absence of Sox3 leads to a derepression of Snail1 expression.

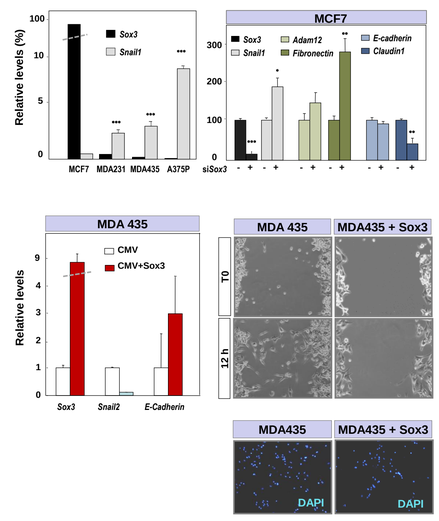


5- Conservation of Sox3/Snail antagonistic relationship in human cancer cells

• Epithelial (MCF7) cells express high levels of *Sox3* and low levels of *Snail1*, as mesenchymal cells (MDA231, MDA435 and A375P) are devoid of *Sox3* with high *Snail1* expression.

•*Sox3* downregulation in MCF7 cells is accompanied by an increase in *Snail1* and mesenchymal markers like *Adam12* and *Fibronectin* while *Claudin1* is repressed.

• **Sox3 expression in MDA435 partially reverse the mesenchymal phenotype.** This is associated with a decrease in *Snail2* expression and an increase in *E-cadherin*. Migratory and invasive behaviors are also affected by Sox3 expression. Sox3 expressing cells do not cover the wound as control cells do in 12 hours and less nuclei of cells that invaded the collagen matrix were stained with DAPI.



CONCLUSIONS

1 Snail2 is sufficient to trigger cell delamination in the epiblast

2 Snail/Sox3 reciprocal repression defines ectodermal versus mesendodermal territories

3 Snail2 and Sox3 are reciprocal direct transcriptional repressors

4 Snail/Sox3 relationship is conserved in mouse embryos and human cancer cells