RESEARCH HIGHLIGHT

An emerging molecular mechanism for the neural vs mesodermal cell fate decision

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Understanding how primary cell fates are established and maintained in the vertebrate embryo provides important insights that inform directed *in vitro* differentiation of embryonic stem cells or adult cells that have undergone induced pluripotency. Neural differentiation is of particular interest as new neural cells may contribute to therapeutic approaches to nervous system injury and diseases and provide *in vitro* disease models for small molecule screening and for determining personalized drug treatments.

As the vertebrate embryonic axis elongates, there is a fine spatial separation of the temporal events of differentiation that facilitates investigation of distinct steps in neural differentiation [1]. At the tail end of the embryo epiblast, cells adjacent to the rostral primitive streak (caudal lateral epiblast, CLE) can give rise to neural or paraxial mesoderm cell fates, depending on whether they become displaced rostrally to form the neural plate or gastrulate through the primitive streak to make paraxial mesoderm (Figure 1A), while some cells are retained in this region (closest to the primitive streak) and act as stem cell-like axial progenitors [2, 3].

A recent paper in *Nature* from Kon-
doh and colleagues has begun to shed

light on the molecular basis of this neural vs paraxial mesoderm cell fate decision [4]. This elegant study uses a series of mouse mutants to explain the homeotic-like phenotype of mice with a homozygous mutation for the T-box family transcription factor *Tbx6*, which possess two ectopic neural tubes in place of the bilaterally arranged paraxial mesoderm [5]. In doing so, Takemoto *et al.* reveal that *Tbx6* acts in part by downregulating a specific enhancer (N1) of the neural progenitor marker *Sox2*.

Unlike other Sox1B genes, *Sox2* expression extends caudally from the neural tube to include the epiblast cells located adjacent to the primitive streak (Figure 1B). Work from this same group in the chick embryo has shown that the apparently uniform *Sox2* expression along the neural axis is in fact composed of several domains, each regulated by a distinct enhancer and responsive to different signaling pathways [6]. Expression driven by the N1 enhancer is found around and flanking the primitive streak and is promoted by FGF and Wnt signaling [4] and inhibited by caudal BMP signaling [7]. These signals focus its activity in the so called caudal neural plate and CLE (epiblast just rostral and adjacent to the primitive streak). In contrast, *Tbx6* is expressed in the primitive streak and newly emerging paraxial mesoderm.

Analysis of the expression of the N1

enhancer-driven transgene encoding *Egfp* in wild-type mice showed that enhancer activity is downregulated as cells transit the primitive streak. However, in the *Tbx6* mutant, enhancer N1 activation persists in the emerging paraxial mesoderm compartment, while the domains of other Sox1B genes are unchanged. This suggests that *Sox2* N1 enhancer activity is responsible for failure to switch off the neural programme when *Tbx6* is lost. This is tested in mouse mutants homozygous for the N1 region of the *Sox2* locus and *Tbx6*, which now lack ectopic *Sox2* expression in the paraxial mesoderm compartment and these mutant embryos fail to make ectopic neural tubes. Importantly, these mice also fail to express mesodermal genes, even though the neural programme is no longer aberrantly active, indicating that *Tbx6* is required both to inhibit N1 activity and to promote mesoderm specification. It will be intriguing to determine what character this non-neural, non-paraxial mesoderm tissue has acquired, as supplementary data indicates it also lacks endodermal markers.

The authors further demonstrate that mis-expression of *Sox2* specifically in the caudal paraxial mesoderm, using a tissue specific enhancer of the gene *Delta1*, is also sufficient to transform presumptive mesoderm into neural tissue. It would be interesting to know now whether this is mediated by *Tbx6* loss?

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Figure 1 Sox2 expression is regulated by Tbx6 via Sox2 enhancer N1. Sox2 expression in paraxial mesoderm is suppressed by Tbx6 via inhibition of enhancer N1. (A) Schematic diagram of the caudal part of developing mouse embryo. (B) Sox2 is normally expressed in the rostral CLE (dashed blue oval) in wild-type embryos. This expression is driven by enhancer N1 as deletion of the enhancer abolishes Sox2 expression in the CLE (C). $Tbx6^{-/-}$ mutation leads to ectopic Sox2 expression in the paraxial mesoderm and this is lost in $Tbx6^{-/-}\Delta N1/\Delta N1$ double homozygous mutant embryos. (D) Illustration of Sox2 expression in transverse sections at indicated levels in (B, C) (dashed lines), predicted from whole mount images in [4]. NT, neural tube; S, somite; CLE, caudal lateral epiblast (dashed blue oval); PS, primitive streak; PM, paraxial mesoderm; NP, neural plate.

The simplest way by which Tbx6 might inhibit N1 activity is by binding to this *Sox2* locus region. However, the authors were unable to establish this and so favour an indirect mechanism of action. A hint of how this might operate comes with the upregulation of *Wnt3a* in the presomitic mesoderm of *Tbx6* mutants, as Wnt and FGF signaling promote N1 activity [4]. The authors propose a model in which during normal development Tbx6 mediates loss of Sox2 enhancer N1 activity in cells leaving the primitive streak via repression of *Wnt3a* expression.

However, this correlation between increased Wnt signaling and increased neural tissue in the *Tbx6* mutant raises some apparent contradictions with respect to other mouse mutants that possess a similar phenotype; ectopic neural tubes in place of paraxial mesoderm. Many of these mutants lack canonical Wnt signaling, see table 1 in [3], correlating low, not high Wnt with a neural

over mesodermal cell fate decision. Similarly, mice lacking the retinoic acid catabolising enzyme *Cyp26a*, which have excess RA [8, 9] that represses *Wnt3a* [10], also form ectopic neural tubes at the expense of mesoderm. In addition, *Wnt3a* is required for *Fgf8* expression [11], and mice in which FGFR signaling is blocked also form neural at the expense of mesoderm tissue (although this is associated with a failure to undergo gastrulation). Surprisingly, Kondoh and colleagues further report no significant change in the expression of *Fgf4* and *Fgf8* transcripts in *Tbx6* mutants [4]. Clearly Wnt signaling is important for both neural and mesodermal differentiation and it will be essential in the future to resolve these puzzling findings and determine the link between Tbx6 and *Wnt3a* transcription. A next step might be to examine readouts of Wnt and FGF signaling and to establish the precise timing and location of any changes in activity levels with respect to the onset

of ectopic N1 enhancer activity in *Tbx6* mutants.

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