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Effect of retinoic acid signaling on Wnt/β-catenin and FGF signaling during body axis extension

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ABSTRACT

Cell–cell signaling regulated by retinoic acid (RA), Wnt/β-catenin, and fibroblast growth factor (FGF) is important during body axis extension, and interactions between these pathways have been suggested. At early somite stages, Wnt/β-catenin and FGF signaling domains exist both anterior and posterior to the developing trunk, whereas RA signaling occurs in between in the trunk under the control of the RA-synthesizing enzyme retinaldehyde dehydrogenase-2 (*Raldh2*). Previous studies demonstrated that vitamin A deficient quail embryos and *Raldh2*$^{-/-}$ mouse embryos lacking RA synthesis exhibit ectopic expression of *Fgf8* and *Wnt8a* in the developing trunk. Here, we demonstrate that *Raldh2*$^{-/-}$ mouse embryos display an expansion of FGF signaling into the trunk monitored by *Sprouty2* and *Pea3* expression, and an expansion of Wnt/β-catenin signaling detected by expression of *Axin2*, *Tbx6*, *Cdx2*, and *Cdx4*. Following loss of RA signaling, the caudal expression domains of *Fgf8*, *Wnt8a*, and *Wnt3a* expand anteriorly into the trunk, but no change is observed in caudal expression of *Fgf4* or *Fgf17* plus caudal expression of *Fgf18* and *Cdx1* is reduced. These findings suggest that RA repression of *Fgf8*, *Wnt8a*, and *Wnt3a* in the developing trunk functions to down-regulate FGF signaling and Wnt/β-catenin signaling as the body axis extends.

1. Results and discussion

During vertebrate embryogenesis, the process of body axis extension begins when somitogenesis commences. As somites form, the body extends along the anteroposterior axis forming a new domain (the developing trunk) located between the headfolds and the epiblast/primitive streak. Several secreted cell–cell signaling molecules control body axis extension including Wnt (Grigoryan et al., 2008), fibroblast growth factor (FGF) (Del Corral and Storey, 2004), and retinoic acid (RA) (Duester, 2008). Some of the actions of Wnt ligands are transduced through stabilization of β-catenin which can then enter the nucleus and bind to the LEF/TCF family of transcription factors (DasGupta and Fuchs, 1999; Logan and Nusse, 2004). During the early phase of body axis extension, Wnt/β-catenin signaling domains are limited to regions on either end of the developing trunk in the headfold and epiblast/primitive streak (Nakaya et al., 2005). Likewise, FGF signaling domains during the early phase of body axis extension are limited to regions on either end of the developing trunk in cardiac mesoderm (Sirbu et al., 2008) and epiblast/primitive streak (Sirbu and Duester, 2006). Although FGF signaling and Wnt/β-catenin signaling pathways play important roles in body axis extension, the mechanisms by which individual FGFs or Wnts control the processes of body patterning are largely unknown.

Interactions between the Wnt/β-catenin, FGF, and RA signaling pathways have been reported in RA-deficient embryos generated either through vitamin A deficiency which removes the precursor of RA, or through genetic loss of retinaldehyde dehydrogenase-2 (*Raldh2*) which controls RA synthesis (Duester, 2008). Loss of RA signaling in vitamin A deficient quail embryos and *Raldh2*$^{-/-}$ mouse embryos up-regulates caudal *Fgf8* expression and results in segmentation defects during body axis extension including a shortening of the body along the anteroposterior axis and somite left–right asymmetry (Del Corral et al., 2003; Vermot et al., 2005). Further studies with *Raldh2*$^{-/-}$ embryos demonstrated that loss of RA signaling results in an anterior expansion of *Fgf8* expression from the epiblast into the posterior neuroectoderm (Sirbu and Duester, 2006), and a posterior expansion of *Fgf8* mRNA and FGF signaling from cardiac mesoderm into trunk lateral plate mesoderm (Ryckebusch et al., 2008; Sirbu et al., 2008); together, these two events reduce the size of the Fgf8-free zone where the trunk initially develops. Loss of RA signaling in *Raldh2*$^{-/-}$ embryos and vitamin A deficient quail embryos also results in ectopic trunk

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expression of *Wnt8a* (avian ortholog is *Wnt8c*), suggesting that Wnt/β-catenin signaling may also be down-regulated by RA signaling in the developing trunk (Niederreither et al., 2000; Olivera-Martinez and Storey, 2007).

Here, we examine FGF and Wnt/β-catenin signaling in mouse *Raldh2*$^{-/-}$ embryos during the early phase of body axis extension. *Raldh2*$^{-/-}$ embryos are completely devoid of RA signaling from E7.5 to E8.5 (0–10 somites) when body axis extension commences (Sirbu and Duester, 2006; Sirbu et al., 2005). As a marker of FGF signaling we examined expression of *Sprouty2* (*Spry2*) which is induced by FGF signaling (Minowada et al., 1999) and acts as a negative-feedback regulator of the pathway (Hanafusa et al., 2002). Whereas *Spry2* mRNA is normally expressed in two separate domains anterior and posterior to the developing trunk, *Spry2* was greatly up-regulated in *Raldh2*$^{-/-}$ embryos such that the two domains are nearly joined in the developing trunk (Fig. 1A and B). We also examined another marker of FGF signaling, *Pea3* encoding an Ets transcription factor induced by FGF (Raible and Brand, 2001), and observed an anterior extension of its caudal expression domain in *Raldh2*$^{-/-}$ embryos (Fig. 1C and D). These findings indi-

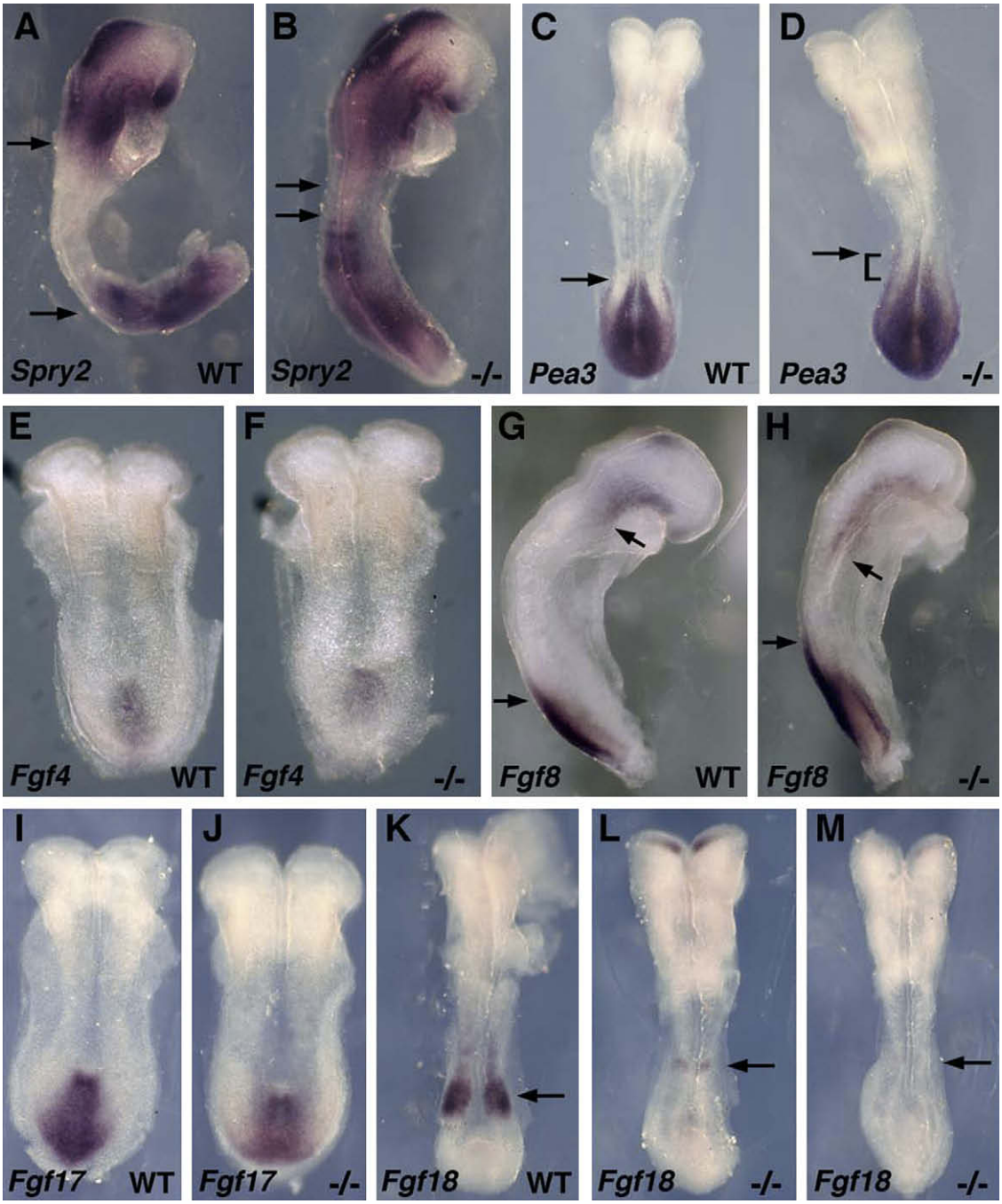

Fig. 1. FGF signaling following loss of RA synthesis. (A and B) *Spry2* mRNA and (C and D) *Pea3* mRNA at the 6-somite stage; arrows indicate that the *Raldh2* mutant exhibits a large increase in *Spry2* expression in the developing trunk, and an anterior extension of the caudal *Pea3* expression domain. (E and F) *Fgf4* mRNA at the 4-somite stage; no difference is observed between wild-type and *Raldh2* mutant embryos. (G and H) *Fgf8* mRNA at the 5-somite stage; arrows indicate that the anterior and posterior domains of *Fgf8* expression extend further into the trunk in the *Raldh2* mutant. (I and J) *Fgf17* mRNA at the 4-somite stage showing no difference between wild-type and mutant. (K–M) *Fgf18* mRNA at the 7-somite stage; arrows indicate that caudal *Fgf18* expression is either lost or greatly reduced in *Raldh2* mutants.