Materials and Methods

Raldh2 null mutant mice (*S1*) and RARE_*hsp68_lacZ* transgenic mice (*S2*) have been described. Whole-mount ISH were performed on embryos fixed in 4% paraformaldehyde and processed as described (*S3*), using an In Situ Pro (Intavis) Robot. ISH plasmids were kindly provided by M. Kmita (*lacZ*), N. Brown (*Lfng*), R. Kelly (*Fgf8*), Y. Saga (*Mesp2*), R. Kageyama (*Hes7*), M. Petkovich (*Cyp26A1*), A. Joyner (*Fgf18*), P. Gruss (*Uncx4.1*), E. Robertson (*Nodal*), C. Goridis (*Pitx2*) and P. Bouillet (*Meox1*). Immunolabelling with a RALDH2 antibody (a kind gift of P. McCaffery) was performed as described (*S4*). Double ISH and immunolabelling were performed using fast red tablets for alkaline phosphatase detection of digoxigenin-UTP labelled RNA probes, according to the manufacturer's protocol (Roche). After stopping the enzymatic reaction by washes in PBS-0.1 % tween 20, immunofluorescence was performed as described (*S4*) using a rabbit polyclonal anti-β-galactosidase (5 prime-3 prime, Inc.). Alexa 488 coupled secondary antibodies were used (Molecular Probes). Embryos were analysed using a Leica Sp2RS confocal microscope or a Leica M420 stereomacroscope.

Supporting text

We have investigated whether the uncoordinated progression of the oscillatory waves in *Raldh2*$^{-/-}$ embryos affects downstream genes transiently expressed during each cycle of somitogenesis. Expression of the *Mesp2* homeobox gene roughly spans the length of one cycle of somite formation (*S5*). Hence, wildtype mouse embryos exhibit two symmetrical *Mesp2* stripes in the rostral PSM (Fig.S4A), or four stripes when a cycle of expression has been induced before complete downregulation in the former somites (Fig.S4B). *Raldh2*$^{-/-}$ embryos exhibited either symmetrical or LR asymmetrical (n=11/27) *Mesp2* patterns. In some mutants, *Mesp2* was expressed as two misaligned stripes, separated by a distance of approximately one prospective somite (Fig.S4C). Other mutants had two stripes on one side and one on the contralateral side (data not shown). These patterns may reflect the progressive delay of somite formation in the right-side mutant PSM. Some embryos had three *Mesp2* bands on the right side and two on the left side (Fig.S4D), further suggesting a delayed maturation of the right-side PSM.

We have also analyzed expression of left-right (LR) molecular determinants in the *Raldh2*$^{-/-}$ embryos. Expression of *Nodal*, an early determinant of the left-right axis and of *Pitx2*, a downstream effector, was analyzed in 4-9 and 7-16 somite-stage embryos, respectively. As described previously (*S6,S7*), *Nodal* expression was inconsistently detected in control embryos (n=29/44), including among specimens of the same somitic stage. A similar fraction (n=12/16) of mutant embryos showed detectable *Nodal* expression. In all instances, expression was specific of the left lateral plate mesoderm (lpm) (Fig. S5A,B). All control (n=30) and *Raldh2*$^{-/-}$ (n=26) embryos showed left-side specific *Pitx2* expression in the lpm (Fig.S5C,D). We therefore conclude that left-right axis specification is statistically not

affected in the *Raldh2* mutants. This result is consistent with the fact that the majority (~ 95%) of the *Raldh2*$^{-/-}$ embryos exhibit delayed somitogenesis on a given side of the embryo. The few mutants showing a reversed somitic phenotype may thus correspond to the small fraction of mouse embryos with a reversed left-right axis.

Supporting figures

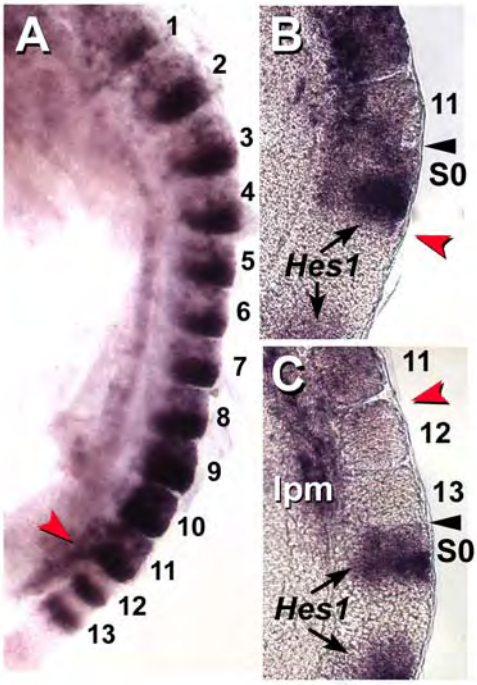

Figure S1. A: Combined detection of *Uncx4.1* and *lacZ* transcripts in a RARE_hsp68_*lacZ* transgenic embryo. Thirteen somite pairs have been formed, as seen from the *Uncx4.1*-labelled stripes (arabic numerals). However, *lacZ* expression (purple signal between the *Uncx4.1* stripes) is only seen until the level of the 11th somite. B,C: Combined detection of *Hes1* and *lacZ* transcripts in two RARE_hsp68_*lacZ* embryos. *Hes1* is an 'oscillating' gene expressed in a striped pattern within the PSM, the most rostral expression stripe corresponding to the somite undergoing maturation (S0) (*S8*). In a 11 somite-stage embryo (B), the RARE_*lacZ* positive domain extends until the *Hes1*-labelled stripe in S0, whereas in a 13 somite-stage embryo (C), both domains are now demarcated by a non-labelled somite. Thus, progression of the RA-responsive front within the PSM stops at the level of presomites 11-12. Red arrowheads indicate the posterior limit of the *lacZ* signal. Black arrowheads in B,C show the last formed (SI/S0) intersomitic boundary.

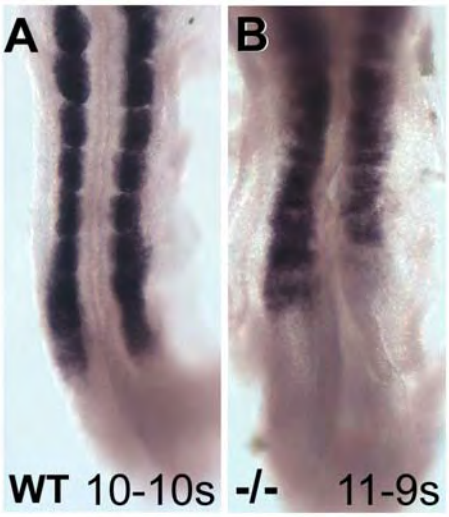

Figure S2. Whole-mount ISH of wildtype (A) and *Raldh2*$^{-/-}$ (B) embryos with a *Meox1* probe (dorsal views). The numbers of formed somites on the left and right sides are indicated below.

Figure S3. Detail of an E8.5 *Raldh2*$^{-/-}$ embryo co-labelled with *Uncx4.1* and *Lfng*. *Uncx4.1* labelling indicates severely delayed somite formation on the right side of the embryo. The waves of *Lfng* expression are uncoordinated in the left and right caudal PSM (arrowheads) and a 'salt and pepper' distribution of expressing and non-expressing cells is seen within the right-side rostral PSM (bracket). This suggests that, in the most severely affected mutants, a

desynchronization of the molecular oscillations occurs on the right side of the embryo, leading to an arrest of somitogenesis.

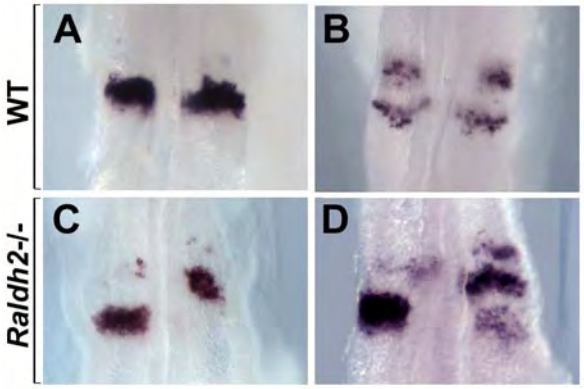

Figure S4. Whole-mount ISH of wildtype (A,B) and *Raldh2*$^{-/-}$ (C,D) embryos with a *Mesp2* probe. Dorsal views (details of the somite-forming region).

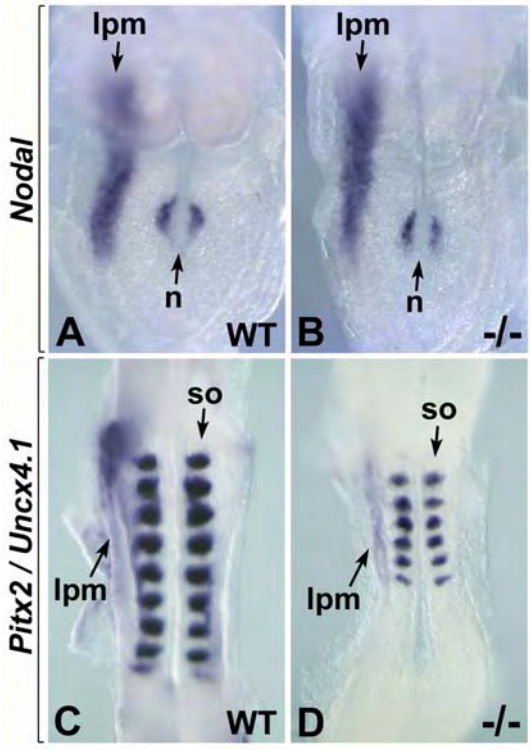

Figure S5. Whole-mount ISH of wildtype (A,C) and *Raldh2*$^{-/-}$ (B,D) embryos with *Nodal* (A,B) and *Pitx2* probes (C,D, double labeling with *Uncx4.1*). Dorsal views. lpm: lateral plate mesoderm; n: node; so: somites.

Supporting references and notes

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S7. J. Collignon J, I. Varlet I, E.J. Robertson. *Nature* **381**,155-8 (1996)

S8. C. Jouve *et al.*, *Development* **127**, 1421-9 (2000).