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. Mc Caffery) 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 stereomacro scope.
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<sup>−/−</sup> (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<sup>−/−</sup> 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<sup>-/-</sup> (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<sup>-/-</sup> (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