Control of Vertebrate Left-Right Asymmetry by a *Snail*-Related Zinc Finger Gene

Alison Isaac, Michael G. Sargent, Jonathan Cooke*

A gene encoding a zinc finger protein of the *Snail* family, *cSnR*, is expressed in the right-hand lateral mesoderm during normal chick development. Antisense disruption of *cSnR* function during the hours immediately preceding heart formation randomized the normally reliable direction of heart looping and subsequent embryo torsion. Implanted ectopic sources of intercellular signal proteins that are involved in establishing normal left-right information randomized the handedness of heart development and also altered the asymmetry of *cSnR* expression. *cSnR* thus appears to act downstream of these signals, or perhaps in parallel with the latest expressed of them, the Nodal protein, in controlling the anatomical asymmetry.

Generally in vertebrates looping of the heart tube and the associated torsion of the embryo axis are the first manifestation of consistent left-right asymmetry, or "handedness," in development. In birds, however, an earlier but transient asymmetry is apparent at Hensen's node, where dorsal axial tissue is generated during gastrulation (*1*). Normal right-handed heart looping is highly conserved throughout vertebrates (*2*), the human incidence of left looping, situs inversus, being around 0.01% and often associated with congenital heart disease (*3*). Cardiac tissue is derived essentially symmetrically from lateral mesodermal regions of the embryo that later fuse in the midline to form a tube (*4*). The precise site and nature of the asymmetrical events that soon cause bending in this tube are unknown (*5*). Four genes encoding intercellular signaling proteins are known to show early asymmetries of expression (*6*, *7*), three of which are implicated from chick and mouse experiments in the developmental transmission of left-right information in the period between gastrulation and heart looping. Here we report another chick gene involved in this expression of handedness, *Snail*-related (*cSnR*), that encodes a putative zinc finger transcription factor.

The *cSnR* gene was isolated in addition to the closely related gene *Slug* (*8*) when a chick cDNA library was probed with a *Xenopus* gene that had itself been homology-cloned with the *Drosophila* gene *Snail*. The *Xenopus*, chick, and mouse genes (Fig. 1) share two regions of amino acid sequence similarity to *Snail*: that containing the zinc fingers and another short one at the NH_2-terminal end. Transcription of *cSnR* starts in the primitive streak but declines to low levels in emigrating mesoderm cells. Transcripts are never observed in Hensen's node, but they occur briefly throughout the presumptive neural ectoderm just at the full-length streak stage. *cSnR* is newly expressed, bilaterally at first, in presumptive anterior cardiac mesoderm as node regression begins (Fig. 2A). In subsequent hours expression in the right-hand domain intensifies, and a wave of expression passes posteriorly through the elongated, laterally situated right cardiac mesoderm territory as this inrolls to meet its left counterpart and form the heart tube (Fig. 2B). The right-hand posterior inflow heart territory continues to express the gene while the anterior, fused and already looped ventricular region has become *cSnR*-negative (Fig. 2, C and I). Meanwhile the gene is up-regulated sharply and bilaterally in the lateral edges of newly segmenting somites and spreads further into the cross section of more mature somites (Fig. 2, B, C, E, and F). It is expressed in mid-ventral pharyngeal endoderm (Fig. 2I) and, at later stages, extensively in posterior lateral and tailbud mesoderm.

We used antisense *cSnR* oligonucleotides (oligonucleotide A, nucleotides 1 through 15; oligonucleotide B, nucleotides 63 through 78; numbered from the G of the start methionine codon). Oligonucleotides were used singly or together at a total concentration of 40 μmol in the incubation mixture. Embryos were incubated with oligonucleotides for 1.5 to 2 hours at the head-process stages [stages 5 through 8 (*9*)], with or without lipofection, before being returned to their vitelline membranes in ring culture (*8*, *10*). Embryos were then examined 18 to 30 hours after treatment, when the normal heart had formed and looped to the right, 10 to 20 somites had segmented, and the basic plan of the nervous system and head had formed. More than 200 such experimental embryos and 150 embryos treated similarly but with control oligonucleotide sequences (*11*) were examined.

Effects specific to antisense *cSnR* treatment included anomalous somite segmentation (*12*) and a 30 to 50% incidence of reversed heart looping (Fig. 3, A and B). Such situs inversus was often associated with a reversal of embryo torsion (*13*). Our conclusion that these effects are the result of specific interference with *cSnR* function is based on the following: (i) The effects were sequence-specific because the majority of oligonucleotide sequences were without effect. (ii) The effects corresponded with unusual features of the

```
      0
XSna  MPRSFLVKKH FSASKKPNYS ELESQTVYIS P.FIYDKFP. ..VIPQPEI
cSnR  MPRSFLVKKH FSASKKPNYS ELESQTVLAA PL.LYETCA. LSVIPPPEVL
mSna  MPRSFLVRKP SDPRRKPNYS ELQDACVEFT FQQPYDQAHL LAAIPPPEVL

     50
XSna  STGAYYTPLV WDTGLLTTFF TSESDYKKSP ISPSSSDDSS KPLDLTSFSS
cSnR  GPGAYYPPLV WDAGLL.... .SSLFPAGLG TEAEAAGGAA PALDLTTLSS
mSna  NPAASLPTLI WD.SLL.... ..VPQVRPVA WATLPLRESP KAVELTSLS.

    100
XSna  EDEGGKTSDP P..SPASSAT EAEKFQCNLC SKSYSTFAGL SKHKQLHC..
cSnR  EEDEGKSSGP P..SPASAPA AARKFRCAQC AKAYSTFAGL SKHKQLHC..
mSna  DEDSGKSSQP PSPPSPAPSS FSSTSASSLE AEAFIAFPGL GQLPKQLARL

    150
XSna  ....DSQTRK SFSCKYCEKE YVSLGALKMH IRSHTLPCVC KICGKAFSRP
cSnR  ....DAQTRK SFSCKYCEKE YVSLGALKMH IRSHTLPCVC KMCGKAFSRP
mSna  SVAKDPQSRK IFNCKYCNKE YLSLGALKMH IRSHTLPCVC TTCGKAFSRP

    200
XSna  WLLQGHIRTH TGEKPFSCTH CNRAFADRSN LRAHLQTHSD VKKYQCKSCS
cSnR  WLLQGHIRTH TGEKPFSCTH CNRAFADRSN LRAHLQTHSD VKKYQCKTCS
mSna  WLLQGHVRTH TGEKPFSCSH CNRAFAVRSN LRAHLQTHSD VKRYQCQACA
```

Fig. 1. Predicted amino acid sequence alignment of proteins encoded by *XSna* (*Xenopus*), *cSnR*, and *mSna* (mouse) from the cDNAs. The GenBank sequence accession number for *cSnR* is Y09905. Domains forming the zinc fingers are underlined, but in *mSna* the first of these domains has diverged so as not to encode a true finger, leaving only four domains. There is 49% amino acid identity for all three reading frames across the whole protein, but for the region of zinc fingers 2 through 5, identity is 89%. Single letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I; Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

*To whom correspondence should be addressed.

gene's expression pattern. (iii) Antisense oligonucleotides to other chick genes have caused quite different whole-embryo effects that are also related to those gene expression patterns (*8*, *14*). (iv) Identical antisense sequences delivered as two different chemical modifications of oligonucleotide DNA were able to produce the same effects (*11*). (v) A reduction specifically in *cSnR* mRNA levels, especially in right-hand lateral mesoderm, was observed at stage 8-9 directly after antisense *cSnR* treatment only (Fig. 3, C and D). (vi) Most critically, unaffected mRNAs after treatment with a combination of A and B oligonucleotides included that of *Slug* (8), whose corresponding target sequences differ from the *cSnR* ones by two bases (oligonucleotide A) and five bases (oligonucleotide B).

The mirror-reversed heart structure and torsion are consistent with the loss of a gene function that imparts handedness to, rather than itself executing, morphological asymmetry. Lateralized *cSnR* expression was still to the right in spontaneous and antisense-induced situs inversus cases from ring culture, suggesting a perturbation of the gene product's effectiveness rather than its site of expression. Situs inversus occurs most often when antisense oligonucleotides are introduced at stage 8+ (four somites), during the period of most pronounced *cSnR* expression asymmetry just before heart tube formation. Treatment at the full-length primitive streak stage a few hours beforehand in development has no effect on either situs or segmentation, consistent with relatively transient antisense attenuation of gene function. The spontaneous incidence of situs inversus in ring culture, although unaffected by control oligonucleotide treatments, is significant (8%) in relation to the rare occurrence in ovo (< 0.1%). It also occurs most when the embryo has been explanted into culture at stage 8+. The execution of morphological asymmetry may be initiated within the right posterior part of the straight stage 9 heart tube (*5*).

We next tried to determine when *cSnR* is expressed within the sequence of left-right asymmetrical gene expression that begins at gastrula stage (*6*, *7*). Near Hensen's node at stage 4, the gene *activin receptor IIa* is locally expressed on the right in ectoderm. Shortly thereafter, *Sonic hedgehog (Shh)* is expressed on the left in the same layer. This sequence may reflect initial up-regulation of an activin-type ligand on the right, which then acts locally through the induced receptor to prevent lateral *Shh* expression. In subsequent hours, extending into the time of heart tube formation, the transforming growth factor–β (TGF-β)–related gene *nodal* is expressed on the left only in mesoderm, first near the regressing node and then in an extended domain including cardiac tissue. This expression may be initiated by the lateralized *Shh* expression. It mirrors the right-lateralized component of *cSnR* expression, though it tends to peak slightly earlier and in slightly more anterior tissue. The relevance of this cascade for developmental asymmetry is supported by results of the implantation of protein-releasing sources near the node of the full-length primitive streak (6). The gene *nodal* can be up-regulated in right lateral mesoderm by ectopic hedgehog protein from a right-side source and down-regulated on the left by a left-side activin source, and both of these manipulations also randomize the direction of later heart looping.

We first incubated stage 7 embryos with antisense *cSnR* oligonucleotides and probed for *nodal* shortly afterward during its normal period of maximal expression. The expression of *nodal* was unaffected after 4- or even 7-hour treatments (longer than those used to randomize heart looping). *cSnR* may thus be downstream of *nodal*, or in a parallel pathway of asymmetry information.

We next studied *cSnR* expression itself

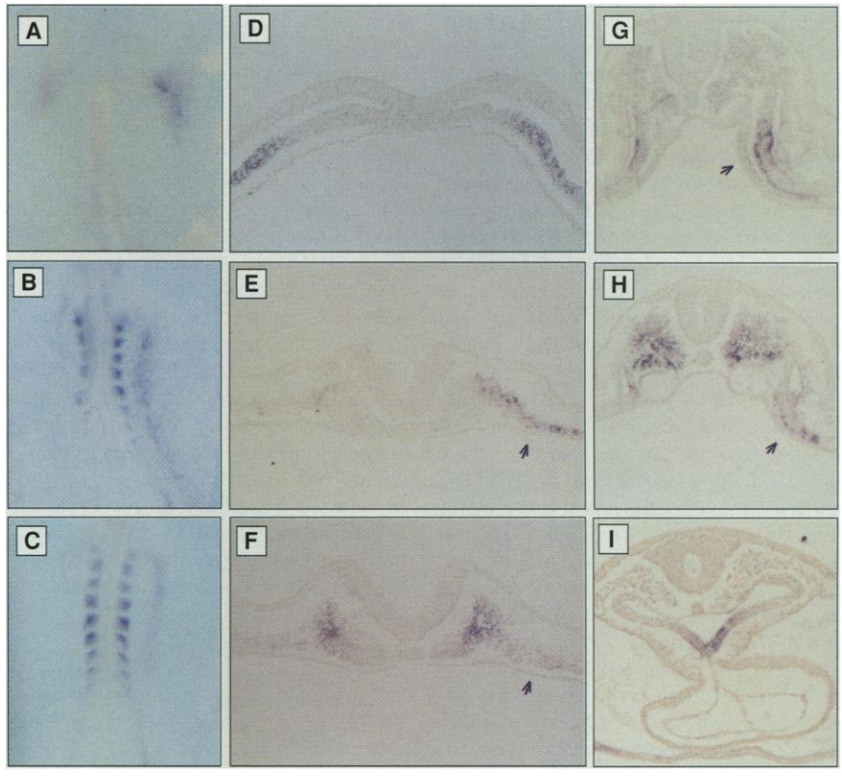

Fig. 2. Pattern of *cSnR* expression analyzed by digoxygenin (DIG) in situ hybridization (*23*). Panels (A) to (C) show whole-mount specimens from a dorsal aspect, anterior at top. (**A**) The early head process stage (stage 5). In this, though not in all, stage 5 specimens, expression in the lateral, presumptive heart territories opposite Hensen's node already appears stronger on the right. (**B**) The 6- to 7-somite stage. Asymmetry of expression in posterior cardiogenic, lateral plate mesoderm is at its most pronounced. Expression is now throughout the first-formed somites, as well as bilaterally in lateral edges of the new somites and in tissue that will form the lateral boundaries of future somites. (**C**) The 10-somite stage. Remaining lateral plate expression is largely confined to the right, inflow heart region. Panels (D) to (I) are transverse sections, looking anteriorly, across in situ whole mounts as shown in (A) to (C). Arrowheads mark regions of higher, right-lateralized expression. (**D**) Heart-forming regions of a stage 5 embryo as shown in (A). Expression, still almost symmetrical in this specimen, is almost entirely in the mesoderm layer. (**E**) Section at about the time of formation of the first somite, between the stages in (A) and (B). (**F**) Relatively anterior section through a 6- to 7-somite embryo showing expression throughout the somites and in the mesoderm destined to enter the right-hand heart tube. (**G**) A more posterior section through the embryo of (F) showing expression predominantly in right splanchnic (posterior heart-forming) mesoderm and adjacent endoderm. (**H**) Relatively posterior section through a 10-somite embryo. Stronger right-hand expression in mesoderm joining the caudal, future inflow part of the heart. (**I**) More anterior section through the embryo of (H). Expression (now symmetrical) is in the endoderm of the pharynx floor, but not within the formed heart tubes.

after placement of beads (*15*) as ectopic sources of activin and hedgehog proteins, to the left and right of the node, respectively. Embryos were cultured for another 12 hours until stage 8+, then fixed and probed for *cSnR* or *nodal* expression. Ectopic expression of hedgehog protein on the right eliminated *cSnR* expression from its normal right lateral domain (Fig. 4G) and up-regulated *nodal* in this same position as reported (*6*). *Shh*, either directly or through activation of *nodal*, could thus contribute to preventing *cSnR* activation in left precardiac tissue of the normal embryo.

In response to the expression of activin protein on the left side, the lateralized *cSnR* expression component was variously perturbed in slightly more than half of the cases, whereas almost one-third of all embryos probed for *nodal* expression showed similar perturbations of this gene (Fig. 4, A through F, and Table 1). The results for *nodal* differ from those previously reported (*6*), and together with those for the *cSnR*, they have several implications (Table 1). In previous studies the beads caused duplication on the left of the gene expression normal to the right, thus depriving embryos of the "handed" information conveyed by certain genes. The activin beads we implanted, however, seem to drive embryos

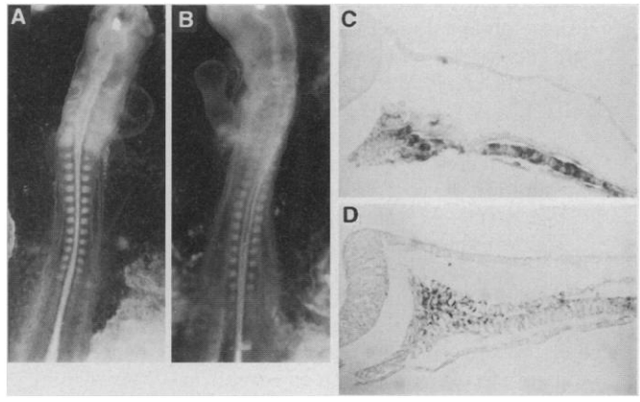

Fig. 3. Antisense *cSnR* oligonucleotide treatment leads to randomization of heart situs. (A) and (B) show dorsal views of stage 12 embryos after incubation with oligonucleotides for 1.5 to 2 hours at stage 7 (*9*). (**A**) Normal heart looping (50 to 70% of antisense-treated embryos in various experiments). (**B**) Situs inversus (30 to 50% of treated embryos). Note the onset of axial torsion. Embryos incubated with control oligonucleotides showed situs inversus in less than 10% of all cases (8 to 13% in various experiments). (C) and (D) are transverse sections at similar levels through the right somite and lateral plate mesoderm of control oligonucleotide- and antisense-treated embryos. The embryos were cultured for 4 to 5 hours with antisense *cSnR* or control mixtures (longer than for typical experiments demonstrating the described phenotype) and washed before fixation at the 6- to 7-somite stage. (**C**) Control-treated embryo showing a normal amount of *cSnR* RNA signal for cultured embryos. Note that lateral *cSnR* expression is specific to splanchnic (heart-forming) mesoderm. (**D**) Antisense-treated embryo showing an overall reduction of the *cSnR* signal. There were a number of individuals in which a reduction to the level illustrated was confined to the lateral expression component.

Table 1. Results of activin bead implantation. After implantation of activin-loaded beads to the left of the node at stage 4 (see Fig. 4), embryos at stage 8 were randomly assigned to analysis by DIG whole-mount in situ hybridization for either *nodal* or *cSnR* expression patterns. For *nodal*, normal expression is on the left side; for *cSnR*, normal expression is on the right side. Numbers indicate embryos expressing each possible pattern of lateral (cardiogenic) expression for each gene. On the hypothesis that lateral expression of *nodal* and *cSnR* is in fact exclusive by this stage, embryos in columns 1 and 2 (normal and reverse side) would then be members of the same class of outcome of bead placement (normal or "reversed" left-right asymmetry), which occurs with particular probability. But embryos in column 3, for each gene, would be equivalent to those in column 4 for the other in that bilateral expression of each gene should co-occur with nonexpression of the other ("double-left" or "double-right" development). On this hypothesis the probability of observing the numbers seen, on probing of two random samples of 60 bead-implanted individuals for each gene, is about 0.15 (χ^2 test; 3 degrees of freedom). Thus, although *cSnR* expression might seem to have been abnormal after the treatment more often than that of *nodal*, the numbers are consistent with the notion that those embryos affected by beads are brought to a condition from which lateralized expression of these genes develops randomly, but with exclusivity.

Gene	Number of embryos with expression on				Total
	Normal side	Reverse side	Both sides	Neither side	
nodal	42	8	6	6	62
cSnR	28	15	9	6	58

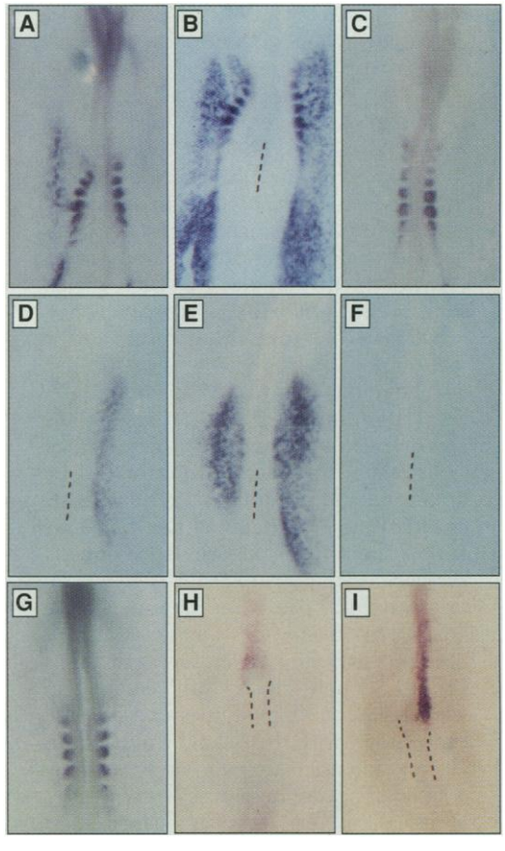

Fig. 4. Bead implantation at primitive streak stage 4. Implanted beads created ectopic, left-hand sources of activin or right-hand sources of hedgehog protein lateral to Hensen's node (*6*). Beads frequently became dislodged during in situ hybridizations, but that in (A) is visible. Embryos probed for *cSnR* or *nodal* gene expression during stage 8-9 (*9*) and midline marked where not structurally visible on photographs. The *Shh* expression pattern at Hensen's node shown in (H) and (I) was probed during stage 6, and dashed lines mark the junction of the anterior streak and node. For incidences of the outcomes shown in (A) to (F) and of unchanged, normal expression of *cSnR* and *nodal* in response to beads, see Table 1. (**A** to **C**) In situ hybridization for *cSnR* after treatment with left activin. In (A), reversed, left expression in lateral plate mesoderm. For normal, right expression see Fig. 1B. In (B), symmetrical expression on both right and left. In (C), absence of expression in lateral plate mesoderm. Note the retention of normal bilateral somite expression. (**D** to **F**) In situ hybridization for *nodal* expression after treatment with left activin. In (D), reversed, right expression. For normal, left expression, see (*6*). In (E), expression on both left and right. In (F), absence of expression. (**G**) In situ hybridization for *cSnR* expression after treatment with right-hand hedgehog protein. Normal right-hand lateral plate expression of *cSnR* is absent, leaving symmetrical somite expression only, as in (C). (**H** and **I**) Embryos probed for *Shh* expression during stages of morphological node asymmetry (*1*). In (H), normal (left asymmetrical) expression. In (I), reversed (right asymmetrical) expression.

backward with respect to the cascade of asymmetry information during development, such that lateralized gene expression is destabilized and may "redevelop" independently on each side. In support of this idea, we observed reversed, right-lateralized *Shh* expression with reversed structural asymmetry (*1*, *16*), and also cases of symmetrically down-regulated lateral *Shh* expression that left midline expression only (*6*) at the regressing node a few hours after implantation of an activin bead on the left at stage 4 (Fig. 4, H and I).

The variables that influence the time course and intensity of ectopic signals from beads are unclear (*15*), but our results are consistent with the idea that when the gene cascade for asymmetry is recovering randomly from perturbation by ectopic activin, *nodal* and *cSnR* cannot stably be coexpressed on the same side in lateral mesoderm because the *Shh-nodal* pathway down-regulates *cSnR*. In normal development, expression of *cSnR* on the right could also be independently activated through the activin pathway. The peak of *cSnR* lateralized expression during stage 8+, when there is peak susceptibility to both antisense- and culture-induced situs disruption, also suggests that, of the genes discussed here, *cSnR* lies closest to the execution of morphological asymmetry. Studies with experimental grafts have suggested the existence of a character on the right that dominates in provision of left-right information, and that becomes autonomously instated in precardiac tissue by stage 5-6 (*17*). This is the period during which pronounced right lateral enhancement of *cSnR* RNA normally commences, consistent with the idea that such expression, even if originally repressible by *Shh-nodal*, may constitute this dominant information once stabilized.

The normal right-sided accentuation of the activin pathway, thus far the earliest appearance of asymmetrical gene expression, cannot itself account for the reliable origin of handed information. Some earlier chiral molecular cue within cells must trigger the normal directionality of a tissue-wide asymmetry system (*2*, *18*). If the original chiral cue is no longer effective, our activin bead–treated embryos may redevelop randomly as either normal, reversed, "double right," or "double left" with regard to lateral mesodermal gene expressions, with the second condition reversing and the last two conditions randomizing morphological asymmetry. Mammal mutants, as yet uncharacterized, show reversed and randomized situs (*19*). But the disrupted genes might lie upstream, closer to the original chiral cue, because *nodal* expression is appropriately altered in each affected individual (*7*).

Further downstream genes remain to be found that may execute asymmetrical morphology itself, perhaps by altering local growth rates or by production of mechanical bending forces (*5*). Because the *Drosophila* protein encoded by *Snail* is thought to be a repressor (*20*), any immediately downstream gene product might be preferentially expressed in the left posterior (inflow) heart tube region. One candidate is the protein flectin (*21*).

The mouse gene *mSna*, although definitely not the homolog of *Slug* (*22*), is probably not the mammalian homolog of *cSnR*. Its protein has only four true zinc fingers and it has a different, symmetrical expression pattern. The partial, but perhaps incomplete, conservation of the early left-right genetic pathway between chick and mouse (*6*, *7*) suggests that identification of any true mammalian *cSnR* ortholog would be interesting.

REFERENCES AND NOTES

1. R. Wetzel, *Verh. Phys. Med. Ges. Wurtsburg* **49**, 227 (1924); J. Cooke, *Nature* **374**, 681 (1995).
2. N. A. Brown and L. Wolpert, *Development* **109**, 1 (1990).
3. J. Torgerson, *Arch. Pathol.* **47**, 566 (1949); M. Campbell and D. C. Deuchar, *Br. Heart J.* **28**, 472 (1966).
4. H. Stalsberg and R. L. DeHaan, *Dev. Biol.* **19**, 128 (1969); H. Stalsberg, *ibid.*, p. 109.
5. N. Itasaki, H. Nakamura, H. Sumida, M. Yumida, *Anat. Embryol.* **183**, 29 (1991); H. Stalsberg, *Am. J. Cardiol.* **25**, 265 (1970).
6. M. Levin, R. L. Johnson, C. D. Stern, M. Kuehn, C. Tabin, *Cell* **82**, 803 (1995).
7. J. Collignon, I. Varlet, E. J. Robertson, *Nature* **381**, 155 (1996); L. A. Lowe *et al.*, *ibid.*, p. 158; C. Meno *et al.*, *ibid.*, p. 151.
8. M. A. Nieto, M. G. Sargent, D. G. Wilkinson, J. Cooke, *Science* **264**, 835 (1994); L. Essex, R. Mayor, M. G. Sargent, *Dev. Dyn.* **198**, 108 (1993).
9. V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1951).
10. For further details on the optimal development of cultured embryos and for the technique of lipofection in conjunction with phosphorothioate oligonucleotide treatment used in most of our experiments, see D. A. T. New, *J. Embryol. Exp. Morphol.* **3**, 326 (1955); J. Cooke and A. Isaac, in *Molecular Embryology: Methods and Protocols*, P. Sharpe and I. Mason, Eds. (Humana, Totowa, NJ, in press). Lipofectamine (Gibco-BRL) was used with lipid:DNA molar ratios recommended as for transfection. All oligonucleotide DNA, chemically modified for nuclease resistance, was supplied by Oligos Etc. (Wilsonville, OR).
11. Oligonucleotide sequences that had no effect at concentrations up to 80 μmol included the following: the corresponding sense ones, those identical with the antisense ones except for internal six–base 5′-3′ reversals, a random sequence mixture (*8*), and several antisense oligonucleotides to chick *brachyury*, *noggin*, and *follistatin* sequence targets. Antisense chick *Slug* oligonucleotides giving the previously reported abnormalities (*8*) had no effect on heart, embryo torsion, or somite development. Phosphorothioate oligonucleotides may act by RNA message degradation or translational arrest, or both [see, for example, K.-H. Schlingensiepen and W. Brysch, in *Gene Regulation: Biology of Antisense RNA and DNA*, R. Erickson and J. Izant, Eds. (Raven Press, New York, 1992), pp. 317–328]. The identical antisense *cSnR* oligonucleotide sequences as *p*-ethoxy DNA, which has different pH and charge properties in our system, give identical specific effects at comparable concentrations. This further supports the evidence that effects are gene sequence–specific, because occasional chemically mediated, sequence-conditioned (but not gene sequence–conditioned) effects of thioated DNAs have been reported
12. Extra fissures divide off lateral bodies, which form their own epithelial somites out of series with the main file, in anterior parts of the somite series. *cSnR* expression is dynamic in and near lateral parts of the nascent somite cross section.
13. After extirpation of heart territories in ring-cultured stage 8-9 embryos (that is, before any macroscopic, structural heart asymmetry has occurred), the axis, which has no heart yet, twists in the normal direction with about normal reliability. This suggests that torsion is potentially an independent manifestation of handed information in embryos. The frequent reversal, delay, and other disturbances to torsion in our antisense *cSnR*–treated situs inversus embryos therefore suggest that the lateral *cSnR* expression component may control torsion too [though see (*5*)].
14. D. Srivastava, P. Cserjesi, E. N. Olson, *Science* **270**, 1995 (1995).
15. We used Affigel beads, because optimal results have been obtained in other work with these as ectopic sources of both soluble Shh protein and the TGF-β (activin)–related bone morphogenetic proteins. We confirmed randomization of heart looping (*6*) using these beads with both *Shh* and activin.
16. We have also incubated whole streak-stage blastoderms for 2 hours in either 20 *Xenopus* animal cap units (2 to 4 ng/ml) of recombinant activin A protein or the activin antagonist follistatin protein (2 μg/ml) (courtesy of the National Pituitary and Hormone Repository, Rockville, MD) before returning them to culture. Such global treatments might be expected to obliterate early asymmetry in the intensity of function in an activin or related ligand-receptor system. Either treatment causes abnormal morphological symmetry in the stage 6 node 5 hours later and, ultimately, embryos with hearts of reduced or unlateralized looping as opposed to randomized handedness. This adds to the evidence that an activin-related pathway is fundamental to the transmission of left-right information during gastrulation.
17. C. Hoyle, N. A. Brown, L. Wolpert, *Development* **115**, 1071 (1992).
18. A. Gierer and H. Meinhardt, *Kybernetik* **12**, 30 (1971).
19. N. A. Brown, A. McCarthy, L. Wolpert, *Development* **110**, 949 (1990); T. Yokohama *et al.*, *Science* **260**, 679 (1993).
20. S. Gray, P. Szymanski, M. Lenne, *Genes Dev.* **8**, 1829 (1994); S. Gray and M. Lenne, *ibid.* **10**, 700 (1996).
21. T. Tsuda, N. Philp, M. H. Zile, K. Linask, *Dev. Biol.* **173**, 39 (1996).
22. M. A. Nieto, M. F. Bennett, M. G. Sargent, D. G. Wilkinson, *Development* **116**, 227 (1992); D. E. Smith, F. Francisco del Amo, T. Gridley, *ibid.*, p. 1033. For the mouse *Slug* homolog, M. G. Sargent and M. A. Nieto, unpublished data.
23. M. A. Nieto, K. Patel, D. G. Wilkinson, in *Methods in Avian Embryology*, M. Bronner-Fraser, Ed. (Academic Press, San Diego, 1996), pp. 219–235.

24. We thank A. Nieto, Instituto Cajal, Madrid, for the complete *cSnR* cDNA; M. Levin and C. Tabin, Department of Genetics, Harvard Medical School, for the chick *nodal (cNR-1)* plasmid; H. Roelink, T. Jessell, and M. Placzek for hedgehog protein; and Oligos Etc. Incorporated for extendeded advice and test samples.

10 October 1996; accepted 7 January 1997