Snail coiling: CRISPR editing of a single gene turns righties into lefties

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Synopsis
The establishment of left-right (l-r) body asymmetry is a key biological process which is strictly regulated genetically. We have demonstrated unequivocally that the handedness of the freshwater snail *Lymnaea (L.) stagnalis* is determined by the single maternal diaphanous gene *Lsdia1*. The gene operates at the one-cell stage to set the chirality and dictates the entire chiromorphogenesis programme across the levels of biological hierarchy. We have also revealed that mechanical micromanipulation during the 3rd cleavage to reverse the spiral-cleavage direction creates mirror-image animals.

Abstract

Most animals display external bilateral symmetry, but the structure and position of the visceral organs show asymmetries that are determined genetically. To reveal the still unknown mechanism of the establishment of left-right body asymmetry, we have chosen to work on the freshwater snail *L. stagnalis*, which has several unique characteristics. 1) Both the dextral and the sinistral strains within a species are found in the wild with 98% of the snails dextral and 2% sinistral. 2) The asymmetry is displayed both externally and internally. 3) Snail handedness depends on the direction of spiral cleavages of the embryos, as was reported as early as 1894.[1)] 4) The snail is a hermaphrodite, and hence both self-crossing and crossing can occur. This makes the genetics more versatile and informative.

Fig. 1 shows three left-right pairs of *L. stagnalis*: **a)** a pair in the wild, **b)** one created by mechanical manipulation and **c)** the sinistral one is created by CRISPR/Cas9 genome editing of the dextral one. Contrary to what has been believed, temporal and spatial cytoskeletal dynamics for the left- and right-handed snails within a species are not mirror images of each other. Thus, during the third cleavage, helical spindle inclination (SI) and spiral blastomere deformation (SD) are observed only in the dominant dextral embryos at metaphase-anaphase, whereas in the recessive sinistral embryos, helicity emerges during the furrow ingression (Fig. 2)[2)]. We have constructed the congenic F10 strain that on average has inherited 99.9% of the sinistral-derived genome and only 0.1% of the dextral strain-derived genome. All the dextral embryos oviposited by F10 animals that inherited the dextrality gene(s) within the 0.1% of the dextral-derived genome exhibited dextrotropical rotation with SD/SI at the 3rd cleavage and without exception grew to possess dextral body shape. Equally, all the sinistral embryos oviposited by F10 animals that do not inherit the dextrality-determining gene(s) showed levotropical rotation without SD/SI, and grew to possess the sinistral body shape,

again without exception. These findings strongly suggested that dextrality is determined by a single gene or by genes at closely linked loci.[3)]

We carried out positional cloning and identified that *Lsdia1* is the strongest candidate gene for the handedness determination. There are tandemly duplicated *Lsdia1* and *Lsdia2* genes whose proteins share 89.4% amino acid similarity. In the sinistral strains, both *Lsdia1* alleles had a frameshift mutation early in the coding region (c.184delC) that leads to protein truncation.[4)] Further, knocking out the *Lsdia1* gene using CRISPR/Cas9 produced sinistrally-coiled offspring generation-after-generation in the otherwise totally-dextral genetic background, if biallelic frameshift mutations had occurred (**Fig. 1c**).[5)] We could show that the gene sets the chirality at the one-cell stage, the earliest observed symmetry-breaking event linked to body-handedness in the animal kingdom. The gene dictates the entire chiromorphogenesis programme across the levels of biological hierarchy wherein the early intra-cellular chirality is superseded by the inter-cellular chirality during the 3rd cleavage, leading to asymmetric *nodal/Pitx* expressions and then to organismal body handedness.[5)]

In parallel, we revealed that mechanical micromanipulation during the 3rd cleavage to reverse the spiral cleavage direction creates mirror-image animals (**Fig. 1b, 2**). The expression site of the *nodal/Pitx* genes was inverted by mechanical micromanipulation. (**Fig. 2**).[6)]

Diaphanous genes are present in all the Eukaryotes, and therefore, our work featuring formin-controlled early onset of chirality in *L. stagnalis* may provide new insight on unifying mechanisms of l-r body plan formation in animals.

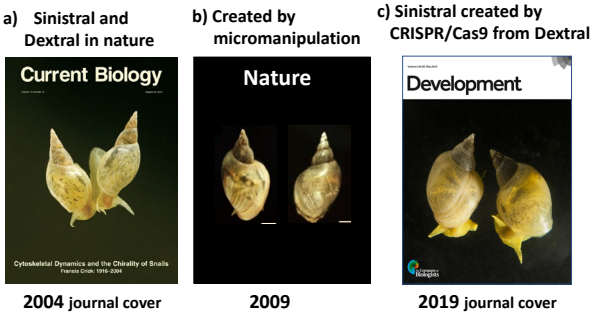

Fig.1 Three l-r pairs in the wild (a) and created (b, c).

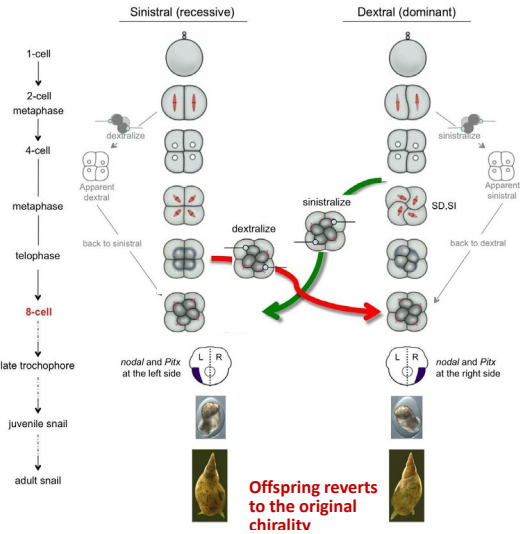

Fig.2. Breaking of mirror-image relationship, and l-r inversion by mechanical manipulation.

References

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