The development of handedness in left/right asymmetry

NIGEL A. BROWN1,* and LEWIS WOLPERT2

1MRC Experimental Embryology and Teratology Unit, St George’s Hospital Medical School, Cranmer Terrace, London, SW17 0RE, UK
2Department of Anatomy and Developmental Biology, University College and Middlesex Hospital School of Medicine, Windy Ridge Building, Cleveland Street, London, W1P 6DB, UK

* Author for correspondence

Summary

The development of handed asymmetry requires a special mechanism for consistently specifying a difference between left and right sides. This is to be distinguished from both random asymmetry, and from those left/right differences that are mirror symmetrical. We propose a model for the development of handedness in bilateral animals, comprising three components, (i) A process termed conversion, in which a molecular handedness is converted into handedness at the cellular level. A specific model for this process is put forward, based on cell polarity and transport of cellular constituents by a handed molecule, (ii) A mechanism for random generation of asymmetry, which could involve a reaction–diffusion process, so that the concentration of a molecule is higher on one side than the other. The handedness generated by conversion could consistently bias this mechanism to one side, (iii) A tissue-specific interpretation process which responds to the difference between the two sides, and results in the development of different structures on the left and right. There could be direct genetic control of the direction of handedness in this model, most probably through the conversion process. Experimental evidence for the model is considered, particularly the iv mutation in the mouse, which appears to result in loss-of-function in biasing, and so asymmetry is random. The model can explain the abnormal development of handedness observed in bisected embryos of some mammalian, amphibian and sub-vertebrate species. Spiral asymmetry, as seen in spiral cleavage and in ciliates, involves only conversion of molecular asymmetry to the cellular and multicellular level, with no separate interpretation step.

Key words: asymmetry, handedness, laterality, isomerism, heterotaxia.

Introduction

The development of handed asymmetry is a deep and neglected problem. Deep because it involves a type of spatial ordering of a quite special nature for which there are just no models, and neglected for, probably, just such reasons. By handed asymmetry we mean consistent left/right differences, such as the dextral looping of the heart in vertebrate development, variations in the number of lung lobes on the two sides in mammals, and the larger habenula nucleus on the left side of the newt brain. Handed asymmetry must be distinguished from both random asymmetry, and from the differences between the left and right sides that arise in bilateral symmetry. Mice develop a preference for the use of one paw rather than the other, this persists throughout life, and Collins (1975) has shown that either the left or right paw may be preferred – the asymmetry is random. The mirror symmetry of many (bilateral) animals results in structures on each side being different from each other. For example, in vertebrates the left limb is clearly different from the right limb, but this does not require any intrinsic difference between the left and right sides during development. The limbs are mirror images of one another – they are completely symmetrical about the midline axis, and thus there is no asymmetry. The developmental processes on the two sides of the midline axis, are, with respect to limb development, identical.

The fundamental feature of handed asymmetry that makes it so distinctive is that it requires a special mechanism for, as it were, distinguishing ‘leftness’ from ‘rightness’. Left and right as properties of spatial pattern differ from the other axes of development in that they are not independent, but are defined with reference to the other axes. The anteroposterior and dorsoventral axes of an embryo may be specified independently, but left and right are defined with respect to both these axes (Fig. 1). Thus, if either the anteroposterior axis or the dorsoventral axis were inverted, the left and the right of the embryo would also be inverted. It is this very dependence on the other two axes that presents the problem. By what means is it possible to specify the left and right of the embryo?

We will argue that one way that this is possible is by
means of a mechanism in which a molecule, which itself has handedness, is aligned with respect to the anteroposterior and dorsoventral axes, and that there is a process we term *conversion* in which this molecular asymmetry is manifested at the cellular and multicellular levels.

In extensive reviews, Corballis and Morgan (1978) and Morgan and Corballis (1978) considered human handedness and cerebral lateralization in terms of the development of other inherited asymmetries (see also Morgan, 1977). They suggested that many asymmetries develop under the influence of a left–right maturational gradient, which external influences can reverse. They further suggested that asymmetry is not directly coded for by genes but that genetic effects were indirect, acting on a basic asymmetry in the cytoplasm. A related model is that of Anett (1978) for the inheritance of human handedness. Essentially, Anett postulates a single gene which biases brain development on the left side, and that in the absence of this gene the asymmetry is random. [See also, McManus and Mascie-Taylor (1974)].

A major influence in both their and our thinking has been the developmental effects of the *iv* (situs inversus viscerum) mutation in the mouse. When homozygous, half the mutant mice exhibit *situs inversus*, that is to say the handed asymmetry of organs is reversed. It is as if a gene necessary for normal handed asymmetry is lost. Layton (1976) and Corballis and Morgan (1978) suggested that the only consistent explanation is that the normal allele does not specify left or right as such, but rather permits the development of a particular handedness. Our interpretation is somewhat different, but we regard the randomness of the asymmetry in *iv/iv* mutants, and in certain other experimental circumstances (see below), as of fundamental importance. In *iv/iv* mice, a handed asymmetry has, by loss of a gene function, been converted to a random asymmetry. It is for this reason that a single process which merely specifies a difference between left and right sides is, by itself, not a sufficient explanation of the development of handedness.

Our model is for the development of handed asymmetry in bilateral animals, but we will also consider its relationship to other asymmetries, including the development of spiral handedness.

### Handedness in bilateral animals – a model

Our hypothesis for the development of handed asymmetry in bilateral animals, such as vertebrates, is that there are three separate processes: (i) a mechanism for converting handedness or asymmetry at the molecular level to handedness at the cellular and multicellular level; (ii) a mechanism for randomly generating asymmetry at the cellular and multicellular level, which can be biased by the mechanism that converts molecular to cellular asymmetry; and (iii) a mechanism for interpreting the asymmetry at the multicellular level so that particular structures develop on one side and not the other.

#### Conversion

The basis of handedness is assumed to be intrinsic to the embryo, and to lie at the molecular level (see below). There must, then, be a mechanism to convert the molecular asymmetry (e.g. the asymmetry present in an optically active molecule) into a cellular and multicellular asymmetry that finally results in the generation of a chemical difference between the left and right sides of an embryo. There are many handed asymmetric biological molecules, and a few cellular structures exhibit handedness, such as microtubules and centrioles. How could such molecular handedness be converted into handedness at the cellular and multicellular level? It is necessary to align such handed molecules or structures in relation to both the anteroposterior and dorsoventral axes, in order to then convert their handedness to specify left and right in the embryo. This is because, as we have discussed, handedness is defined with reference to both anteroposterior and dorsoventral axes and so any conversion from molecular to cellular levels, to make one side consistently different from the other, requires the handed molecules to be oriented with respect to the other two axes.

No models for the conversion of molecular to multicellular handedness have been put forward. We have constructed a model to show how, in principle, such a conversion could be achieved.

Consider a sheet of cells in which the anteroposterior axis, dorsoventral axis, and plane of bilateral symmetry have already been established (Fig. 2). We propose that the cells are polarized with respect to the midline, so that some organelle or protein is more concentrated at that side of the cell farthest from the midline. (This could, for example, be the result of the diffusion of a morphogen produced by midline cells). The unequal distribution of cellular constituents in relation to polarity is well established in a variety of systems, such as epithelia and early stages of nematode development (Schierenberg, 1989). Imagine now an asymmetric molecule in the shape of an 'F' which is held in a specific orientation with respect to both the anteroposterior and dorsoventral axes. One possible mechanism for orienting the F molecule is that it inserts in the ventral cell membrane and has an external domain which binds to extracellular fibres aligned along the anteroposterior axis, the F molecules being oriented towards the...
The development of handedness

Anterior end. Evidence for the possibility of such a mechanism for orienting molecules comes from studies on *Tetrahymena* (see Spiral asymmetry, below). Thus, on both sides of the midline, a series of F molecules will be aligned with respect to both axes (Fig. 2). In this way, the mirror symmetry across the midline, with respect to this molecule at least, is lost.

To establish a left/right difference we propose an interaction between the oriented F molecule and the polarity of the cells with respect to the midline. For example, if the F molecule caused transport of some other molecules in the direction of its 'arms', by, for instance, initiating microtubule assembly, then the transport would be towards the midline on the left side and away from it on the right side. Since the cells are already polarized with respect to the midline, there is now a clear difference between all the cells on the left side compared to those on the right. The 'F' transport is in the same direction as the increased concentration of the polarising constituent on the right side, whereas it is in the opposite direction on the left side (Fig. 2). This difference could easily be amplified.

We propose that some stable property is acquired by right side, perhaps as the result of the interaction of the two (polarising, and F-transported) molecules. We could speculate on the exact nature of the stable property (e.g. gene activation), but the important points are that it is developmentally stable, and that it interacts in some way with the random generation of asymmetry (see below).

Speculative as this model is, it has the virtue of showing how, in principle, conversion could work. It also illustrates that the handed molecule must be oriented with respect to both anteroposterior and dorsoventral axes.

We have assumed an intrinsic molecular basic for handedness, but there have been other proposals. Huxley and de Beer (1934) suggested an electric current flowing down the midline in an anterior to posterior direction. Such a current would produce a magnetic field that could produce a force in opposite directions on the left and right sides. If cellular constituents were sensitive to magnetic fields, then these constituents would become distributed in opposite directions with respect to the dorsoventral axis and, by arguments similar to those used for the previous model, could establish a consistent difference between left and right sides. While there is no evidence for either class of model, we prefer the molecular alternative, as it is more consistent with the known properties of cells.

There is little indication that extrinsic signals, for example from the maternal uterus in mammals, play a role in the development of asymmetry. Mammalian embryos develop normal asymmetry in culture, and we have shown that mutant iv/iv mice (see below) develop abnormal asymmetry, even when transferred at the pre-implantation stage into normal, wild-type, mothers (Brown et al. 1990).

We have assumed that the final distinction between left and right sides will be manifest as some chemical difference, as has been suggested by a variety of workers for many years (reviewed by Huxley and de Beer, 1934). It may be possible that the asymmetric development of structures like the heart and gut could be due, not to chemical differences between left and right, but to mechanical asymmetry, perhaps generated by asymmetry in molecules of the cytoskeleton (J. Lewis, personal communication). However, it is most implausible to invoke such a mechanism for the asymmetry of brain structures like the habenulae, or for lung lobes, or the coelom in sea-urchins (see below). Moreover, it is hard to see how reversal of handedness could occur from mechanical asymmetry.

Random generation of asymmetry

It may be thought that a mechanism of the kind just described could, on its own, account for handed asymmetry. However, there is good evidence that in the absence of 'instructions' for handed asymmetry, random asymmetry develops and this requires a mechanism. Credit for the concept of random asymmetry should go to Wilhelm (1921) (see below) and is strongly supported by the iv mutation in the mouse.

We can think of this asymmetry in terms of assigning, randomly, a property 'X' to one side or the other. Since X must normally develop on either side, but not both, a mechanism is also required to suppress it on one side and allow it to develop on the other. Corballis and Morgan (1978) recognized this and proposed an asym-
metrical gradient of maturation, coupled with inhibition. We favour a simpler mechanism that could be provided by a gradient in a morphogen, as originally proposed by Kauffman et al. (1978), for the specification of compartments in Drosophila. This mechanism is based on reaction-diffusion, and it can effectively divide a tissue into two regions, the concentration of the morphogen falling in one half and increasing in the other. No further inhibitory mechanism is required. If the reaction occurred across the midline plane of symmetry in an embryo, left/right asymmetry would be established. Most importantly, the mechanism is, in principle, random. Almirantes and Nicolis (1987) have shown how a slight initial asymmetry could bias a reaction-diffusion mechanism of this general type to one side rather than the other (Fig. 3). In our model, the slight initial asymmetry is provided by the left/right differences arising from the conversion process described above, thus giving a consistent handedness to the system, in normal circumstances.

**Interpretation**

By interpretation we mean the process by which cells act upon the information given to them that they are on the left or right of the embryo. interpretation of the biased asymmetry is, in principle, evenhanded. Given a difference between left and right sides, there is nothing in the model that favours structures developing on the left rather than the right hand. For example, if the biased asymmetry provided an increased concentration of some substance on the left compared to the right side, the cells of the embryo remain free to interpret this difference. In other words, there is no 'dominance' of one side over the other. Thus, the stomach, spleen and aortic arch of mammals are on the left side, but the right lung has four lobes, the left only one.

**Genetic control of handedness**

It has been argued that genes do not directly control the direction of handedness (Morgan, 1977; Morgan and Corballis, 1978). In support of this contention, it is stated that it is very difficult to find examples where mutation results in 100% reversal of asymmetry. In our model, genes could directly control the direction of handedness, most plausibly at the level of conversion.

It seems unlikely that the basic molecular handedness (the 'F' molecule) could be reversed by genetic variation. For example, at the level of protein structure, it is very hard to imagine a mutation altering the handedness of an alpha-helix, which is almost universally right handed (Galloway, 1987; 1989). Again, at the level of protein assembly, such as in a microtubule, it is very difficult to imagine a genetic change reversing the handedness, since assembly depends upon the three-dimensional conformation of the tubulin monomers, and probably involves interaction between, say, 20 amino acid residues. So, the possibility of genetic control reversing molecular handedness seems unlikely.

However, it is easy to imagine two structural variants of the F protein which became oriented in the opposite direction. For example, the binding site on F for extracellular fibres could be modified so it no longer held the molecule as F, but as â. This would reverse asymmetry not by any change in molecular handedness, but by reversing the conversion process. In support of the direct control of the direction of handedness, Policansky (1982) has shown quite clearly a genetic basis for head rotation in flatfish. It is conceivable that the kind of mutation required to produce such a change in the F molecule would be a rare event. It seems likely that most mutations affecting the conversion process would lead to a loss of function, for example an inability of the F molecule to bind to the membrane, or to transport material. This would lead to absence of handed asymmetry; random asymmetry, rather than a reversal of asymmetry.

In our model, interpretation and the random generation of asymmetry would also be under genetic control. However, for these processes it is difficult to imagine a genetic change that would lead to complete reversal of handedness, although, again, loss of function might lead to anomalies in asymmetry (see below). In our terms, the iv mutation in the mouse must be affecting either the conversion process itself, or its interaction with the random generation of asymmetry. As will be discussed below, we favour the latter interpretation. If some aspect of the interaction between multicellular asymmetry and the random generation of asymmetry were absent, the biasing effect would be lost and asymmetry would be random.

The model can now be considered in relation to some experimental studies.

**Experimental studies**

**Vertebrates**

There is a long tradition of experimental manipulations that have reversed the asymmetry of handed structures in amphibians. Spemann, Meyer and Mangold [reviewed by Wehrmaker (1969) and Oppenheimer (1974)] all carried out operations that resulted in
reversal of the viscera – *situs inversus* viscerum. In conjoined twins of *Triturus*, resulting from mechanical constriction of embryos, the viscera were normal in the left twin, but often reversed in the right. In their twinning experiments, Spemann and Falkenberg (1919) found that 50% of the right twins gave *situs inversus* and Wilhelmi (1921) was the first to suggest that this might be due to chance. She suggested that when the influence of the biased curvature on the left side was removed, then the curvature would be random. There are similar observations in mammalian conjoined twins (see below).

Our model provides an explanation of these observations quite naturally, and for the first time. We suggest that the conversion process results in the acquisition of a stable property in cells of the right half, but not the left (see above), and that this property is involved in the biasing of the random generation of asymmetry. If an embryo is bisected after this process is complete, then the left half is labile, conversion can occur again, new left and right sides can be specified, and development of this half is normal. In contrast, the whole of the right half is already fixed with the stable property and can not be reprogrammed by a second round of conversion. Thus, the two sides of the embryo that develop from the right half do not differ in this property, there is nothing to bias the generation of random asymmetry, and asymmetry develops randomly. It follows that if an embryo is bisected before conversion has taken place then each half should develop normally. Separation of *Triturus* at the two-cell stage (Mangold, 1921) results in both twins being normal - presumably the right side is not yet fixed at this stage. Similarly, mammalian embryos split at early cleavage stages develop normally, as do the vast majority of human monozygotic twins.

The natural occurrence of reversal of handedness in *Triturus* is quite high – around 2%. One of the most surprising ways of increasing the incidence of reversal in amphibia is to simply remove the archenteron roof and replace it, the incidence then being increased to about 40%. This operation, we suggest, might completely destroy the biasing mechanism resulting in almost random asymmetry. Surprisingly, the operation also affects the asymmetry of the spiracle and the habenula.

The dextral looping of the heart is one of the best studied features of asymmetry in vertebrate development. It is the earliest manifestation of asymmetry in the mammalian embryo. Slightly later in development, there is a handed rotation of the embryo. It is interesting that the human, chick, mouse and rat all rotate towards their right side [our observations on the rat are contrary to the report of Deuchar (1971); Brown (unpublished)]. Unfortunately, not enough is known about the development of either of these asymmetries to provide useful insights into the three processes we have proposed. (For reviews see Stalsberg, 1970). The development of heart asymmetry is viewed as a late manifestation of earlier processes in which the asymmetry was established. Support comes from the differences in rate of contraction on left and right sides that can be demonstrated when tissues are explanted before heart looping (Satin et al. 1988).

The *iv* mutation in the mouse is of major importance for the study of handed asymmetry. It was first isolated by Hummel and Chapman (1959) and then characterized by Layton (1976), who showed it to be inherited as a single-gene autosomal recessive trait. Layton hypothesized that the normal allele at the *iv* locus exhibits complete dominance and control of normal asymmetry and that, in the absence of this control, asymmetry is randomly determined. The proportion of *iv/iv* mice with *situs inversus* is 50%, regardless of genetic background. There is no correlation with *situs* of the parent, and the proportion has remained at 50% over more than 30 years. This strongly supports the suggestion of random asymmetry. The most straightforward explanation, in our terms, is the loss of the conversion process. However, the effects of the *iv* mutation are more complex than just random determination of *situs*. There are numerous heterotaxias, isomerisms and heart malformations, which need to be considered in terms of our model. Heterotaxia is the non-concordance of handedness in different organs of the same individual. For example, an animal with aortic arch and heart apex positioned to the normal side (left), but the stomach and spleen positioned on the opposite side to normal (right). This surprising phenomenon is discussed separately below.

An important feature of the *iv* mutation is that, in a significant number of homozygous animals, there is symmetry of normally asymmetric structures (so-called isomerism). For example, instead of four lung lobes on the right side and one on the left, in about 10% of *iv/iv* mice there is either one lobe on both sides, or four lobes on both sides (Brown et al. 1989). These two isomerisms occur at equal frequency, in both *situs inversus* mice and those with normal visceral *situs*. This breakdown of asymmetry needs special consideration. In terms of the model, it implies a breakdown in the process generating random asymmetry. Thus, both sides may have either a high concentration or a low concentration of the putative morphogen that generates random asymmetry.

One interpretation is that the *iv* mutation acts on the mechanism for randomly generating asymmetry. The primary effect of the mutation is to prevent response to the bias, resulting in the observed 50/50 (random) asymmetry; a secondary effect being the occasional generation of high or low concentrations on both sides. Another possibility is that in wild-type animals the interaction of the random generation of asymmetry with the bias provided by conversion is required to ensure that the property 'X' (see above) is expressed on one side and not the other. In the absence of a functional bias, as in *iv/iv* mice perhaps, symmetrical expression of 'X' can occur.

Since normal heart development requires an initial asymmetric looping, and subsequent interaction of asymmetric left and right primordia, it is possible that the frequent occurrence of heart malformations in *iv* mutant mice (Layton, 1978) may also reflect the absence of asymmetry in some of these animals.

Recently, we have studied the development of
handed asymmetry in chimeras made by aggregating \textit{iv/iv} and wild-type 8-cell stage mouse embryos (Brown et al. 1990). Comparison of embryonic handedness with tissue analysis of genotype showed that neither \textit{iv/iv} nor wild-type cells were phenotypically dominant. There appeared to be a partial ‘rescue’ of \textit{iv/iv} by wild-type cells, suggesting a role for cell interactions in the development of asymmetry. However, our observations could not exclude an alternative explanation of cell autonomy. Interestingly, the handedness of heart looping seemed to be correlated with \textit{iv/iv} cell contribution to both the heart, itself, and the visceral yolk sac.

\textit{Situs inversus} is relatively rare in man, occurring at a frequency of 1 in 10 to 20 thousand. The human equivalent of the mouse \textit{iv} mutation may be the Ivemark syndrome (McCusick, 1987). In this syndrome, as in the mouse mutant (see below), not all the organs need have the same handedness and there are many cases of symmetrical organs (isomerisms) such as two left-sided (two-lobed) or two right-sided (three-lobed) lungs. Another group of human autosomal recessive defects, the immotile cilia syndromes, can also be associated with abnormalities of handed asymmetry (Palmblad et al. 1984). Those cases with \textit{situs inversus} are termed Kartegener's syndrome. Isomerisms have also been reported in Kartegener's syndrome, but, while there are similarities with Ivemark syndrome and with the \textit{iv} mutation, the fundamental causes must be different. The association between immotile cilia, which is due to loss of dynein arms, and \textit{situs inversus}, led Afzelius (1976) to suggest that cilia were involved in the development of handedness. Although this may be the case, cilia are normal in Ivemark syndrome (McCusick, 1987) and in the \textit{iv/iv} mouse (Handel and Kennedy, 1984).

It is of great interest that, like the experimentally bisected \textit{Triturus} embryos, observations of conjoined human twins show that whereas the visceral \textit{situs} of the left twin is normal, the asymmetry of the right twin is often abnormal, showing \textit{situs inversus}, isomerism or heterotaxia (Siebert et al. 1989). Although there is not sufficient information to show that the \textit{situs} of the right conjoined twin is random, these findings do imply that similar mechanisms are involved in human development.

Correlation of asymmetries (heterotaxia)

The \textit{iv/iv} mutant mouse allows an important question to be answered: how closely correlated are the handed asymmetries of different organs, when the biasing mechanism is not functioning properly? In their original paper on the \textit{iv} mutation, Hummel and Chapman (1959) reported that, although the \textit{situs} of visceral organs was usually correlated with one another, the association was far from complete. For example, the handedness of the thorax could be completely discordant with that of the abdomen. Our own observations confirm this, and show some discordant handedness of organs in 30\% of adult \textit{iv/iv} mice, and up to 50\% of fetuses (Brown et al. 1989). In general, while asymmetries are correlated, they are not tightly linked. Even at the early stages of development (10 days embryonic age), the direction of rotation of the embryo may be discordant with the direction of heart looping (Brown, unpublished).

Similarly, in \textit{Triton} (see Oppenheimer, 1974), abdominal inversion is not always accompanied by reversal of the heart and great vessels. There is even a graded series of visceral inversions. In the chick, the embryo undergoes torsion at its anterior end so that the head turns and the embryo lies on its left side. When Lepori (1967) divided the unincubated blastoderm, twins developed whose axes of orientation were very varied. About 10\% rotated in the wrong direction of which one third also had \textit{situs inversus}. \textit{Situs inversus} occurred in 3\% of all the embryos and only one had normal torsion. Thus, torsion and heart asymmetry need not have the same handedness, but are usually coupled.

The surprising conclusion from all these observations is that, in the absence of the normal biasing system, it seems that the mechanism for randomly generating asymmetry operates almost independently for each structure of the embryo. In contrast, in animals with a normal biasing system, asymmetries are very highly correlated. Thus, biasing seems to be a single ‘global’ event, as in the acquisition of a stable property proposed above, but the generation of random asymmetry may be a multiple ‘local’ process.

Nematodes

There are a number of asymmetries in \textit{Caenorhabditis}, and these are reflected in asymmetric lineages during development (Sulston et al. 1983). The first indication of asymmetry can be seen at the 6-cell stage, when the future right-hand side lies slightly posterior to the left-hand side. The asymmetry is not laid down at a specific site in the egg since inversion of the drosophontal axes (which would lead to reversal of left and right sides) at the two-cell stage still results in normal development (Priess and Thompson, 1987). The implication is that asymmetric molecules must be (re-)aligned with respect to the new axes.

An analysis of lineages has given rise to the idea of homologous and non-homologous analogs on the two sides of \textit{C. elegans} (Sulston, 1983). Pairs of precursors are analogous in that they generate approximately the same groups of cells on the left and right sides of the animal. Posteriorly, they are homologous, that is they come from similar lineages at the same level, whereas anteriorly there are non-homologous analogs that are derived from different developmental programmes on the two sides. Some lineages involve migration across the midline, with cells adopting similar fates on one side to their sisters on the other. Others will make asymmetrical contributions to midline structures. All these differences, in our terms, reflect reinterpretation of asymmetry, which is probably established at the 6-cell stage. Surprisingly there are, as yet, no mutants that completely reverse asymmetry, but several cases of \textit{situs inversus} have been observed, which is visible in \textit{C.}}
The paired claws of the lobster are different; one is a major (crusher) claw and the other a minor (cutter) claw, and their development provides a nice example of randomness. During early stages of development, the paired claws are symmetrical in appearance and differences only become manifest at quite late stages. The handedness of claws is random. Removal of one claw causes the contralateral one to become the major claw. In the absence of a suitable substratum, both claws develop the minor character (Govind and Pearce, 1989). In the snapping shrimp, loss of a minor claw results in its regeneration, but loss of a major claw results in regeneration of the minor form and the transformation of the intact minor claw in the major form (see Govind and Pearce, 1989). Section of the nerve to the major claw will result in a similar transformation (Mellon and Stephens, 1978). In these cases, random establishment of asymmetry is reversible and is maintained by an inhibitory mechanism.

Insects

If \textit{Drosophila} had a well-defined handedness this might provide an ideal system for genetic analysis. Unfortunately, this is not the case. Smith and Sondhi (1960) analyzed the genetic control of ocelli in the head region and found that while there was variation in the asymmetrical distribution, selection could not modify the distribution so that they had a consistent handedness. The folding of the wings, i.e. which wing is laid on the other, is random (Purnell and Thompson, 1973) as is the distribution so that they had a consistent handedness. Selection could not modify the distribution so that they had a consistent handedness. A striking similarity to the behaviour of vertebrate embryos bisected at an early stage of development and strongly suggests a universal mechanism is involved.

\textbf{Spiral asymmetry}

Spiral cleavage, which occurs in many invertebrates, is the earliest marker of handedness in any system. The egg divides such that the cleavage plane is oblique and this results in the spiral arrangement of the blastomeres. Spiral cleavage does not necessarily imply that asymmetrical structures will develop in either the larvae or adult: most larvae of spirally cleaving animals are bilaterally symmetrical, and so too are the adults. Nevertheless, spiral cleavage is the earliest known manifestation of handedness and the mechanism remains unknown. Of particular interest is spiral cleavage in gastropod molluscs, where the handedness of the cleavage is related to the handedness of their spiral shells.

The development of spiral asymmetry only requires a mechanism for converting molecular asymmetry to the cellular and multicellular level. There is no necessity for an interpretation step because the asymmetry pervades the entire organism, rather than being expressed as differential development on two sides. The direction of coiling is determined by the maternal genotype, a single gene being involved (Sturtevant, 1923; Boycott and Diver, 1923). The dextral gene is regarded as being dominant, but there are some complications due to the appearance of dextral offspring in sinistral lines, some of which breed true. Modifier genes have been invoked to explain this anomaly (Diver and Andersson-Kotto, 1938), and more recently, a sophisticated cross-over model has been proposed (Freeeman and Lundelius, 1982). Since the genetic data show a maternal effect, the cytoplasm of the egg is the most obvious location of the origin of handedness. It is of great interest that Freeman and Lundelius (1982) were able to reverse the coiling of sinistral snails by the injection of a small amount of cytoplasm from dextral eggs. The converse injection had no effect. Their interpretation of this experiment is that the sinistral snails lack a protein that converts a sinistral pattern into a dextral one.

Ciliates, too, have a well-defined spiral handedness, their organelles being arranged in a particular order about the long axis of the cell. Global mirror-image phenotypes of \textit{Tetrahymena} have been studied by Nelsen and Frankel (1989). While there is global mirror-image reversal in left-handed cells, the asymmetry of ciliary rows is not inverted, and they maintain the same asymmetry as in the normal right-handed forms. The global asymmetry of left-handed forms is non-genic but is transmitted through the cytoplasm (Nelsen et al. 1989). These observations suggest that there are two separate systems controlling asymmetry in the cell. One controls global asymmetry and may be thought of as a set of positional values going round the cell. It is the order of the set of positional values that is reversed in the left-handed phenotype and this may be likened to the random generation of asymmetry in bilateral animals. The second system involves the ordering of the cell organelles with respect to the long axis of the cell and may be similar to the process of conversion in spirally cleaving eggs.
Conclusions

Our model has teased-out what we believe to be three quite different processes in the development of handed asymmetry. The distinction between conversion, random generation of asymmetry and interpretation, is important if sense is to be made of experimental results, and new experiments planned. The proposed model for conversion from a handedness at the cellular level to handedness at the cellular and multicellular level is the first of its kind. It is probably only just plausible, and we have no doubt that others will now be encouraged to construct much better mechanisms. Even so, our concept of conversion can account for some old observations of the reversal of symmetry quite satisfactorily and it enables one to think about genetic control in concrete terms. The similarity in the development of left and right halves of bisected embryos of amphibians, sea urchins and humans suggests that a universal mechanism may be involved. We find it encouraging that our model can provide a natural explanation for these observations. Direct genetic control of the direction of handedness is possible at the level of conversion, without alteration of the basic molecular asymmetry. Genetic changes could lead to loss or inactivity of the key molecule, resulting in random asymmetry. There is no basic left or right handedness from the point of view of morphology, no ‘dominance’ of one side, each particular case will depend on conversion and interpretation.

The recent mapping of the chromosomal location of the iv mutation in the mouse (Bruckner et al. 1989) should lead to the isolation of the gene and provide possible insights into the mechanism. Our prediction is that the gene is involved in the interaction of the random generation of asymmetry with the conversion process that should be giving the bias. Our model further predicts that if the mechanism of conversion were understood it would be, in principle, possible to make a transgenic mouse whose asymmetry was consistently a mirror-image of the normal mouse. Meanwhile, it is necessary to design experiments that will illuminate all three processes: conversion, random generation of asymmetry, and interpretation.

We are indebted to many colleagues for critical advice, in particular: J. Frankel, J. Slack, J. Cooke, P. Lawrence, C. Chothia, M. Bretscher, J. Lewis, J. Herberg, and M. Morgan. We are grateful to The Wellcome Trust for financial support.

References

The development of handedness


(Accepted 20 February 1990)