That's What its All About:
Genomic Hokey-Cokey[1] and asymmetry in divergent cellular developmental trajectories and body sidedness.

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My intention is to develop the idea that asymmetry in the spatial organisation of genomes and the mitotic spindle following cell division can offer a mechanism of bifurcation in the subsequent developmental pathways of organisms.
The process of symmetry-breaking is one of the essentials of the emergence of *form* in both living and non-living objects from sub-atomic to cosmological scale. The principle can equally be applied to behaviours and learning as to structures. From one human perspective it is what separates our elegant body forms and those of many of our fellow creatures from symmetrical featureless footballs. Symmetry breaking starts in a significant way very early in our development when a formless symmetrical blob of cells (morulla) derived from the fertilised egg, acquires asymmetry in three senses or planes; dorsal ventral (back- front), anterior posterior (head – tail) and lateral (side to side, left – right), and then progressively transforms into a recognisable animal embryo. Front – back asymmetry is easy to recognise in a body, as is head – tail but left-right asymmetry is less obvious and requires that we look into a mirror since left –right asymmetry is also talked about as mirror symmetry. If we take a nicely tailored left glove and observe its reflection in a mirror it will look like a right glove as we can check by looking at our right hand in front of the reflection (in the same front to back orientation), but no amount of forcing will get that glove onto the right hand (which is another way of saying that right and left gloves are mirror images of one another which are not super-imposable). It might seem that this left-right asymmetry is just a trivial consequence of the establishment of the other two asymmetries but it is unlikely to be as simple as that especially when we consider that later in development there is further symmetry breaking imposed on the bilateral right – left mirror symmetry (eg in mammals the heart is more on the left and the liver more on the right; different sides of the brain are associated with different activities).
For me this implies that cells in the act of proliferating and differentiating into structures (on a trajectory) need to have some intrinsic or extrinsic reference as to which side of the body they will form or occupy and that this may be instrumental in setting up the other axes . This was brought home to me emphatically when I learned that it is estimated that 25% of identical twins are apparently mirror images (known as mirror twins) of one another with respect to left-right asymmetries. That is to say that non-symmetrical skin markings, finger or hair whorls can be used to distinguish the twins one from another as mirror images. They also display higher frequencies of discordance for other asymmetries than would be expected from the frequencies of asymmetry in the general population (Levin). Remembering that identical (monozygotic) twins derive from a splitting of very early stage embryos, that is, in the morulla stage after a few divisions of the fertilised egg

[1] Hokey Cokey, or Hokey Pokey is a popular singing dance for groups of children and others in which the dancers' limbs and other body parts, right then left are sequentially shaken all about. It is seen by some as a mocking parody of the gesticulations and incantations of the Catholic sacrament *Hoc est enim corpus meum* (translation:This is my body) becomes hocus pocus, hokey pokey, hokey cokey

cell (zygote), then one might be forgiven for thinking that the basis for left-right asymmetry is likely to be established very early in embryogenesis, before the other asymmetries, perhaps even within the single fertilised egg itself. There is some experimental evidence for this from studies of mouse embryos in which it seams possible to trace a lateral asymmetry back to the zygote. (ref see Beddington and Robertson 1999). In fact these authors suggest that a bi-lateral asymmetry may be established by an inevitable asymmetry within the single celled zygote, that being the asymmetry established at the site of expulsion of the second polar body which is known as the animal pole. More recently Plusa et al (2005) have provided evidence from sophisticated microscopic analysis of the polarity of the first division coupled to cell fate mapping studies, that bilateral asymmetry in the first division is a determinant of which cells in the gastrula contribute to the embryo proper and which the extra-embryonic structures, arguing strongly for the influence of early asymmetries on the development of form. At the same time Beddington and Robertson (1999) do point out that the experimental production of chimeric embryos[2] could be viewed as arguing for the contrary, suggesting that development of embryonic axes is plastic and not determined by prior irreversible symmetry-breaking events in the zygote. Nevertheless simpler organisms than viviparous mammals where the formation of embryonic axes is not complicated by the material formation from the zygote of an array of extra-embryonic structures, there is a strong indication that symmetry-breaking can be initiated at the first cell division. A prime example is that of molluscan embryos where the sense of asymmetric divisions give rise the snails with left handed or right handed spiral shells (see Paralingula). In yet simpler organisms such as Fucus it is clear that asymmetry is established in the zygote via polarised external signals prior to cell division (Quatrano). Such extrinsically determined systems are beyond the scope of the model here proposed.

I want to propose an alternative hypothesis for symmetry-breaking which is intrinsic and is dependent upon several features of the inevitable asymmetry (mirror symmetry) of daughter cells of the first or second division of the zygote, coupled to the phenomenon of genomic imprinting. It requires also that we conceive of genomes as ordered spaces which embody spatial constraints and are thus capable of asymmetries in their own right. That is to say in the language of asymmetries that they can be assigned chiral identities. The principle of genomic order in terms of the disposition of chromosomes within the nucleus was first proposed by Rabl from direct microscopic observation. He noticed that the middles of chromosomes (centromeres) seem to be tethered at one end of the cell nucleus and ends (telomeres) at the other giving rise to the notion that chromosomes under normal circumstances are arrayed in a stable though not necessarily static configuration (now known as the Rabl configuration)
Subsequent studies employing three-dimensional reconstructions of electron microscopic serial sections of nuclei (Bennett) and more recently chromosome painting (Bickmore) reinforce this notion and go so far as to define ordered domains within genomic space. For a full review see Spector 2003. Furthermore, the observations of Plusa et al (2005) show that maternally and paternally derived chromosomes occupy distinct domains in an

[2] Chimeric mice can be produced by the dissection and fusion of embryonic cell masses derived from different zygotes. One could expect that established lateral asymmetry might constrain this process

arrangement which is retained in daughter cells of the first division of the mammalian zygote.

The principle that the inevitable mirror symmetry of the mitotic spindle could give rise to right and left chiral identities in daughter cells follows from theoretical considerations and observations of dividing tissue culture cells by Albrecht-Buelher (1977)[3] . However, he looked to asymmetry among attendant cytoskeletal fibres as the basis for a chiral identity rather than the configuration of genomic space itself. In figure 1 I reproduce his original figure in which only two pairs of chromatids make a contribution to the asymmetry. This representation is readily converted to represent the core of my hypothesis if an additional pair of chromatids replaces the pyramidal molecule. In a model which relates to Albrecht-Buelher's thinking for the prime determinant of right or left embryonic laterality, Brown and Wolpert postulated a tethered chiral molecule. None of these investigators had the benefit of current knowledge of the internal dynamics and configurations of genomic space and so did not have the opportunity of extending their models in this direction.

The model I want to put forward in the context of the establishment of asymmetric cell divisions as for example in the determination of the embryonal axes is as follows:

1. the two daughter cells of the first division (and/or possibly the second) of the zygote respectively, as a consequence of mirror symmetry of the mitotic spindle, contain a genome, configured as a set of spatially ordered chromosomes of the left or right chiral identity. This chirality extends only to the spatial disposition of whole sets of chromosomes not to the tiers of directional internal coiling of the chromosomes themselves.
2. During the further development of the two daughter cells various embryo-autonomous gene functions are triggered in the switch from maternal identity to embryonic. These require interactions between dispersed loci on chromosomes. Some of these interactions will be constrained or promoted by the chirality of one cell or the other leading to divergence in gene expression.
3. this will lead in turn via divergent expression of small RNAs and concomitant histone and DNA modification to right or left imprinting of the genome of that cell and its descendents . Imprinting will lead to further divergence of right and left lineages and the production of right or left specific gene activities at appropriate stages in development.

A merit of this model lies in its espousal of Occam's Razor in that it is asymmetry in a genomic process itself which can support the imprinting of heritable (in terms of cell lineages) divergent developmental trajectories. It is a geometrical/topological necessity. No polarising external signals, no asymmetric partitioning of intrinsic effectors, no conjectural asymmetrically tethered chiral reference molecules need be invoked. Its special conjecture lies in the requirement for inter-chromosomal contacts and the selective hindrance or facilitation of these by one or other of the chiral configurations of the genome.

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[3] "one may begin to look for a relationship between the universally found bilateral symmetry of organisms and the mirror symmetry between certain daughter cells at an early state of an embryo. Right or left handedness may also be the simplest way by which nature primes one of the daughter cells if only one of them is supposed to enter a certain pathway of differentiation"

Support for the first element of this conjecture comes from an interesting example of divergent cellular trajectories in the immune system. T helper cells of the CD4 category undergo a transformation from a so-called naïve state into Thelper1 (Th1)or Th2 cells which are primed to act in promoting an immune response to intracellular (Th1) or extracellular (Th2) invaders. At its simplest, their role is to present antigens derived from the invader to B cells, which are responsible for producing antibodies, such that clones of B cells appropriate to the challenge are stimulated to propagate and deal with the challenge. Th1 and Th2 appear to follow mirrored trajectories in their patterns of gene expression and are genetically imprinted to retain their trajectory independent of external stimuli see Bix et al 2005 . Proper functioning of the immune system requires that there be a continuous production of Th1 and Th2, but one which can react flexibly to the challenges encountered. It has been shown that a single cell division can mark the transition from naïve CD4Tcell to Th1 or Th2 and Reiner (2003) has put forward the suggestion that this division is an asymmetrical one providing daughter cells one of which is primed to respond to the signals driving it to produce an expanded clone of Th1 cells and the other and expanded clone of Th2 cells. This would be consistent with opposing genomic chiralities of daughter cells providing the priming event to channel extrinsic signals into the stable (epigenetic) imprinting of the two cell types. The interesting feature of the transition from naïve Tcells to Th1and Th2, is a change in the observable interchromosomal interactions involving effector gene loci on chromosomes 10 and 11.(Flavell). Whether or not the chiral identities of naïve Thelper daughter cells forms part of the mechanism for divergent development of Th1 and Th2 cells it is clear that inter-chromosomal interactions do play a part . This is evidence in support of the first part of my conjecture. The second part i.e. that inter-chromosomal interactions can be influenced by chiral differences in the deportment of chromosomes remains conjectural.

For me this model is compelling because it embodies a highly deterministic spatial component based on a geometric inevitability . As I have pointed out previously (Hughes 2005) large-scale sequencing projects and the lust for patenting of precious genomic componentry have seduced us into thinking of genomes as strings of DNA in which resides the only key to the determination of developmental trajectories. Perhaps the consideration of inevitable spatial, sequence-independent determinants such as genomic chirality could redress the balance.

Caveat:

It is not my intention to suggest that genomic chirality is the basis for all instances of symmetry breaking in cell division. Asymmetric cell division is common particularly in plant development where there are fewer opportunities for symmetry breaking and anisotropy via cell mobility (Scheres and Benfey 1999). Numerous models have been put forward to explain symmetry breaking on the basis of unequal partition of cellular components or the asymmetric imposition of external signals. This new model is intended to complement rather than replace such models which, I have to admit, do seem persuasive for many instances of symmetry breaking. The new model merely offers a possible explanation in those cases where the initiation of asymmetric behaviour is obscure.

Of course this essay is inevitably selective with the data especially so given the impossibility of a single author being able to assimilate all of the relevant knowledge in fields as diverse and complex as embryology , immunology, twinship and topology. A single misconception on my part in any of these fields will be sufficient for the model to fall and quite correctly be ridiculed.

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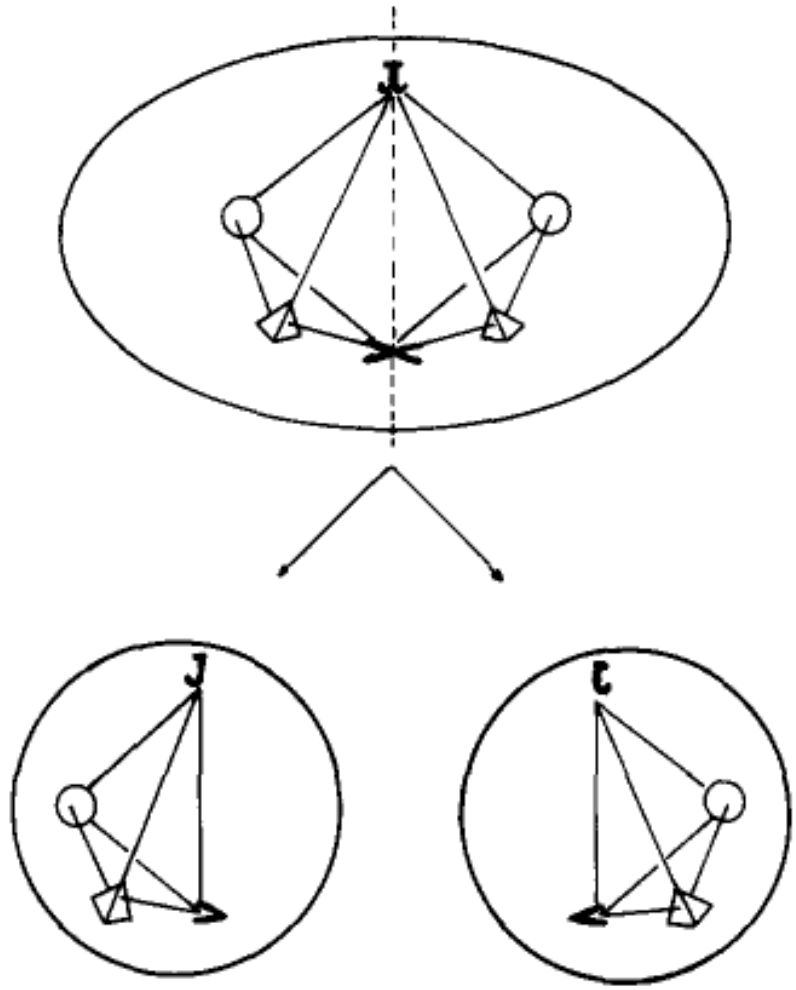

FIGURE 1 Schematic of the way in which mitosis may produce mirror symmetrical intracellular organizations between the two daughter cells. V's and J's symbolize sister chromatids, the small spheres centrioles, and the small tetrahedron represents an unspecified cytoplasmic component. After cleavage, one daughter cell has a right-handed, the other a left-handed, three-dimensional intracellular organization.