

Letter to the Editor

SYMMETRY BREAKING IN CULTURED MAMMALIAN CELLS

Dear Editor:

The generation of natural forms, such as characteristic tissue patterns, involves a process of symmetry breaking in which structures with an initially uniform configuration spontaneously transform into structures with lower symmetry order (Ball, 1999). Symmetry breaking has been examined in biological systems, including formation of fruiting bodies in slime mold (Palsson and Cox, 1996), pattern formation in bacterial colonies (Budrene and Berg, 1991), and generation of animal coat patterns (Murray, 1993); however, the length scale of the characteristic patterns analyzed were orders of magnitude larger than the individual cell components that drive patterning. Here we describe a simplified *in vitro* model in which symmetry breaking and self-organization can be visualized within small groups of mammalian cells migrating within a geometrically confined adhesive space on micropatterned culture substrates created with a microcontact printing technique (Chen et al., 1997).

Development of mammalian tissues, such as capillary blood vessels, is mediated by coordinated migration of cells, which, in turn, is controlled by cell interactions with soluble morphogens, other cells, and insoluble extracellular matrix proteins, such as fibronectin (FN). To create a minimal *in vitro* model of migration-dependent pattern generation, capillary endothelial cells were cultured in medium containing motility factors (basic fibroblast growth factor + 10% serum) on small ($\leq 50 \times 50 \mu\text{m}^2$) FN-coated adhesive islands that were surrounded by nonadhesive barrier regions. When two cells were cultured on a single island, the high symmetry order associated with the ordinarily random movement of cells on unconfined adhesive surfaces (Dunn and Brown, 1987) changed spontaneously. This transition resulted in directional cell migration and lower symmetry order, such that the entire multicellular system synchronously rotated about its geometric center (Fig. 1A). Each cell in this dynamic pattern was led by a lamellipodium that wrapped around the trailing edge of the neighboring cell, thereby creating a sigmoid cell–cell interface reminiscent of the Yin–Yang (YY) symbol of Far Eastern religions (Capra, 1991) (Fig. 1B–D).

The self-organization of stably rotating cellular ensembles was a robust property of the system and not a random transient phenomenon: YYs were always found to be present when the two-cell systems were analyzed and, once the pattern established itself, the cells rotated continuously over the entire period of observation

(over 24 h). YYs were observed to rotate with similar angular velocity in groups of two to four cells cultured on islands of different size (30–50 μm) and shape (circle versus square) (Fig. 1A–D), with equal probability of clockwise or counter-clockwise migration. Emergence of this stable pattern in a group containing only two cells represents the simplest example of symmetry breaking in a mammalian cell population.

A computer simulation based on an idealized minimal model in which two cells migrated on a circular track driven by random walk (Dunn and Brown, 1987) suggests that correlated migration similar to that we observed will spontaneously arise and be stable against perturbations if the normally random motility of these cells exhibits a certain minimal value for two basic parameters: persistence and dynamic coupling (Fig. 2). Persistence is the tendency of a cell to keep moving in the same direction; dynamic coupling indicates that one cell has an increased tendency to move in the same direction as its neighbor when the cells touch (e.g., due to mechanical induction of polarized motion, Verkhovskiy et al., 1999). Endothelial cells exhibit persistence values that are many-fold higher than those of other migrating cells, such as leukocytes (Stokes et al., 1991) and fibroblasts (Ware et al., 1998), and thus, the robustness of correlated migration we observed in endothelial cell populations is in agreement with the model. In fact, fibroblasts did not form YYs when cultured on the same FN-coated islands, and instead showed a straight cell–cell boundary (Fig. 1E).

The transition from random cell motility to coordinated directional migration of cellular ensembles in this simplified *in vitro* system represents a novel class of self-organization at the multicellular level. Such a process generates patterns with characteristic features at the same length scale as the underlying components, namely, the individual cells, as is typical for tissue microarchitecture. This mechanism thus differs from the classical models based on reaction-diffusion (e.g., Turing model) which explain long-range (macroscopic) patterns (Murray, 1993) but averages out local inhomogeneities. Instead, our experimental model incorporates local adhesive and structural cues that are also known to play a key role in mammalian tissue morphogenesis (Huang and Ingber, 1999). Thus, it may provide a useful tool to identify how structural cues may lead to symmetry breaking and the emergence of complex tissue patterns through collective behavior among different cellular and molecular components.

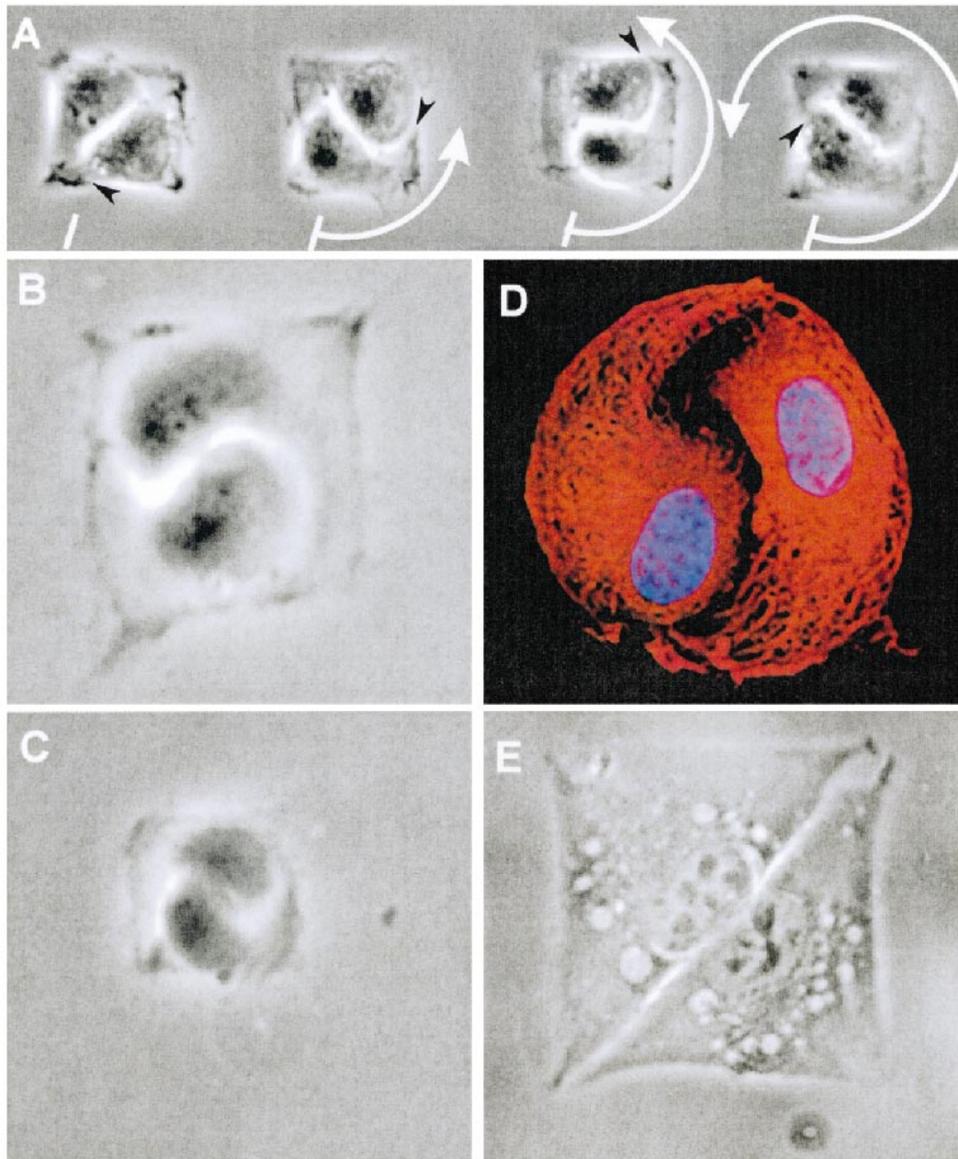


FIG. 1. Spontaneous symmetry breaking within migrating mammalian cells in vitro. (A) Phase contrast images of the same ensemble of two capillary cells migrating in a stable YY pattern for over 1 h on a square FN-coated adhesive island ($40 \times 40 \mu\text{m}^2$). *White arrow* indicates the progressive counter-clockwise movement of the cells; tip of *black arrowhead* abuts on the leading edge of one cell. Similar YY patterns were observed in cells on 50 (B) and 30 (C) μm squares and on circular islands (D); the latter cells were stained for microtubules (red) and nuclei (blue). (E) Two 3T3 fibroblasts on a $50\text{-}\mu\text{m}$ square fail to exhibit YY pattern and rotation. (Note: A movie of the dynamics of Fig. 1A can be seen at: <http://www.childrenshospital.org/research/ingber/movie.qt>.)

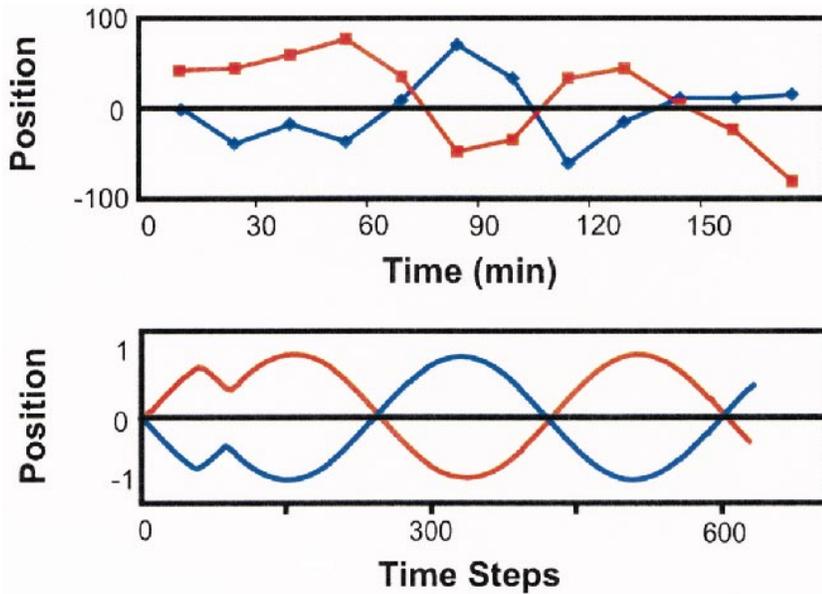


FIG. 2. Comparison between experimentally observed and computer-simulated transitions into the coordinated YY rotation. Experimentally determined changes in the position of each cell on an island plotted as a function of time (*top*) compared with results of a computer simulation (*bottom*). Vertical axis indicates the position of the centroid of the nucleus of each cell projected on an axis of the motion plane (arbitrary units). Note that after an initial period without coupled migration, cells in both the experiment and the simulation began rotating with a consistent phase shift of 180° . The discrete probabilistic model used was based on a biased one-dimensional random walk with persistence, $P = 0.33$, and coupling, $C = 0.33$ ($P = 0$; $C = 0$, represents a completely unbiased random walk. $P = 1$; $C = 1$, indicates complete persistence and coupling, respectively). Uncoordinated movement of cells was observed for values of P and $C < 0.3$.

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