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'Generic' physical mechanisms of morphogenesis and pattern formation

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Summary

The role of 'generic' physical mechanisms in morphogenesis and pattern formation of tissues is considered. Generic mechanisms are defined as those physical processes that are broadly applicable to living and nonliving systems, such as adhesion, surface tension and gravitational effects, viscosity, phase separation, convection and reaction–diffusion coupling. They are contrasted with 'genetic' mechanisms, a term reserved for highly evolved, machine-like, biomolecular processes. Generic mechanisms acting upon living tissues are capable of giving rise to morphogenetic rearrangements of cytoplasmic, tissue and extracellular matrix components, sometimes leading to 'microfingers', and to chemical waves or stripes. We suggest that many morphogenetic and patterning effects are the inevitable outcome of recognized physical properties of tissues, and that generic physical mechanisms that act on these properties are complementary to, and interdependent with genetic mechanisms. We also suggest that major morphological reorganizations in phylogenetic lineages may arise by the action of generic physical mechanisms on developing embryos. Subsequent evolution of genetic mechanisms could stabilize and refine developmental outcomes originally guided by generic effects.

Key words: pattern formation, morphogenesis, genetic mechanism, generic physical mechanism.

Introduction

Developing, regenerating, healing and neoplastic tissues undergo changes in form and cellular composition by mechanisms that are poorly understood. While all contemporary approaches assume that tissue morphogenesis and position-dependent cell differentiation (pattern formation) are caused ultimately by the interplay of physicochemical behaviors of macromolecules, such behaviors fall into at least two distinguishable categories. Certain developmental processes depend on highly organized interactions between specific macromolecules, and can be appropriately characterized as 'molecular machines'. The existence of each such machine presupposes the coevolution of several biological macromolecules, leading to the coordination of their physicochemical properties in the service of a particular function. Examples include cytoplasmic 'motors' that affect the shape and motility of individual cells (Vale, 1987), and gene promoter elements sensitive to complexes of spatially distributed DNA-binding proteins (Štanojević *et al.* 1989; Goto *et al.* 1989).

But there is also evidence that physical forces and dynamical processes that are not the products of the evolved coordination of macromolecular properties, but are organizing principles of nonliving as well as living systems, such as gravity (Ancel and Vintemberger, 1948; Malacinski, 1984), adhesion (Steinberg, 1978; McClay and Ettensohn, 1987; Armstrong, 1989), diffusion (Crick, 1970) and interfacial tension (Steinberg, 1978; Heintzelman *et al.* 1978), participate in important ways in morphogenesis and pattern formation. In contrast to molecular machines, which are mainly suited to bringing about precise outcomes in spatially localized tissue regions, some of these general physical effects may act globally, so as to influence tissue shape and composition over relatively long distances.

Because both highly evolved biomolecular processes (conveniently referred to as 'genetic') and more broadly applicable ('generic') physical processes can each contribute to any given developmental episode, investigators need to take both categories of phenomenon into account. But research on generic morphogenetic and patterning processes is a rapidly expanding area of physical chemistry that is unfamiliar to most developmental biologists. This has restricted the influx of a number of fruitful concepts into developmental biology and impeded the use of several informative cell-free experimental models of morphogenesis.

In what follows we will attempt to redress this deficiency by presenting a typology of generic physical mechanisms relevant to animal tissue behavior. These mechanisms include familiar physical effects such as gravity, viscous flow, phase separation and adhesion. But they also include such exotic processes as Marangoni effects, convective fingering and chemical concentration waves. Previous applications of some of these

mechanisms to development will be reviewed, and additional examples will be given of developmental processes that may profitably be analyzed in terms of such mechanisms.

In our discussion, the morphogenetic properties of individual cells – e.g. their extensibility, contractility and motility – are treated as given; they are assumed to arise from the physical chemistry of highly evolved intracellular proteins such as tubulin, actin, myosin and kinesin, in the presence of sources of metabolic energy and appropriate cofactors. In this sense they are 'genetic'. Similarly, the ability of cells to undergo differentiation in response to microenvironmental signals, and to produce and secrete specific macromolecules, is assumed. Secreted macromolecules will be considered here only insofar as they can potentially play the role of dynamical components in some of the generic physical processes that we will describe. And whereas eggs and multicellular embryos have the ability to produce transcellular ion currents and endogenous electrical fields that reflect morphogenetic polarity, growth and regeneration (Jaffe, 1981; Nucitelli, 1984), it is not clear that bioelectricity as a generic phenomenon has a role in developing systems, apart from its association with transport and utilization of specific ions. We will therefore tentatively group these phenomena with other active chemical processes, and refer the reader to the reviews cited above for further details.

Our main purpose is to familiarize developmental biologists with the range of generic physical mechanisms that can participate in biological morphogenesis and pattern formation, and to indicate possible relationships between these processes and the highly specific molecular interactions that also mediate developmental events and are responsible for the precision of their outcomes. In particular, we suggest that many morphogenetic processes may have first arisen in evolution by the action of generic physical mechanisms on cells and tissues, and that particularly favorable results were later stabilized and made more dependable by the superimposition of more evolved genetically determined mechanisms. In this perspective, the *de novo* origin of developmental mechanisms becomes less problematic: contemporary molecular mechanisms could have evolved as reinforcements for less precise generic physical determinants, the conditions for which may or may not currently prevail. And while the possible generic origins of certain morphogenetic processes may be obscured by an overlay of evolved genetic mechanisms, they can potentially be brought to light by well-conceived experiments.

It will also be noted that many of our biological examples relate to events involving the extracellular matrix. This is not because generic physical processes are prohibited from occurring intracellularly. Indeed, cytoplasmic reorganization in the amphibian egg may make use of such processes (see below). However, it seems reasonable to assume that the constraints on molecular processes within the cell are in general greater than those in the extracellular milieu, and the tolerance of variability correspondingly smaller. Extracellular matrices, under this assumption, would have less of the character of 'molecular machines' than most intracellular macromolecular assemblages, and would therefore be more typical loci for the physical processes we have termed 'generic'.

Examples of Generic Processes

The mechanical properties of materials are conveniently described in terms of their responses to *stresses*, which are forces applied to bodies of matter. A change in the dimensions of a body produced by a stress is called a *strain*. *Shear stresses* act tangentially to planes within the material and cause continguous parts of the body to slide past one another. In solids, shear stresses are opposed by bonds between adjacent subunits and by *elastic* restoring forces, whereas liquids begin to flow as soon as a shear stress is applied. The capacity of a liquid to flow is due to the ability of the liquid's molecules or other subunits to readily change their relative positions.

The physical state of a living tissue can span the range from liquid (blood) to solid (bone). But it is only in intermediate state, semisolid tissues that developmentally significant, short-term morphogenetic effects can take place. Typical tissues exhibit both elastic properties, which permit them to resume their shape when a shear stress is removed, and *viscous* properties, in which rearrangement of internal components (cells or extracellular matrix materials) permits shape change in response to shear stress (Phillips and Steinberg, 1978; Steinberg and Poole, 1982).

Viscoelastic fluids can be compressible or noncompressible; that is, their volume will decrease or remain unchanged under *compressive* stresses, which are forces directed normal to planes within the material. However, tissues are generally noncompressible because of their high water content. Even when local reductions of extracellular space occur, as in mesenchymal tissues undergoing condensation (Thorogood and Hinchliffe, 1975), retention of water will ensure that the overall tissue volume is conserved.

Like other fluid systems, tissues are subject to the ubiquitous effects of gravity and adhesion. Either of these forces can effect shape change, but the degree of deformation will depend on the mechanical properties of the particular tissue (its relative elasticity and viscosity).

The nonuniform distribution within a tissue of mechanical properties, such as density or viscosity, or of chemical components, can be sources of morphogenetic change or cellular pattern formation. However, nonuniformities in density, viscosity or chemical composition will tend to dissipate by mixing unless *either* the different subregions are immiscible, *or* the nonuniformities result from an active, free energy-consuming process that maintains the nonequilibrium heterogeneous state. The latter can only occur in systems that are open to external sources of fuel and raw materials, conditions well-satisified by living tissues. The possibility of maintenance of spatially nonuniform chemical

composition in a tissue by the coupling of biosynthesis and diffusion (Turing, 1952; Prigogine and Nicolis, 1967; Nicolis and Prigogine, 1977) is discussed in a later section.

The formation of immiscible *phases* can preserve spatial heterogeneity even without the expenditure of free energy. Phases are physically distinct, mechanically separable portions of matter in a single heterogenous physical system. Oil and water are a familiar example of distinct fluid phases, but cytoplasm and yolk in amphibian eggs interact in a similar fashion. In fact, as we will describe below, tissues from different sources often behave as distinct fluid phases. Their immiscibility can result from differences in strengths of homotypic and heterotypic cell interactions (Steinberg, 1978), or by virtue of organizational properties of their extracellular matrices (Forgacs *et al.* 1989) (see below).

Immiscible fluid phases are characterized by a tension at their common interface. This interfacial tension represents the incremental increase in free energy brought about by attempting to deform the interface at constant temperature, pressure and composition. A zero interfacial tension is tantamount to miscibility of the subregions. The relative balance of the cohesive interactions within each phase and the adhesive forces between them defines the interfacial tension, as well as determining the contour and extent of the boundary between the regions. Quantitative modulation of the strength of adhesion at the boundary between two liquid phases, and at the boundary of each phase with any tissue surface with which it comes into contact, can induce subtle or gross morphogenetic rearrangements. The relevance of these 'wetting' (de Gennes, 1985) or spreading effects to the behavior of living tissues will be discussed below.

Morphogenetic changes driven by local disparities in interfacial tension are well-known in nonliving systems. Droplets of fluid along the interface are driven in the direction of increasing tension, and the resulting shear stresses induce motion in the bulk of the interfacing fluids. An example is the 'tears of strong wine', in which finger-like streamers form at the air–liquid interface in a wine glass. These phenomena are collectively referred to as Marangoni effects (Scriven and Sternling, 1960; Napolitano, 1984), and may also be relevant to tissue morphogenesis.

Differences in density, viscosity or composition between subregions in a heterogenous particle suspension or multicomponent fluid (and, by implication, a tissue or egg), even in the absence of interfacial tension between the regions, when subject to force fields like gravity or pressure, may give rise to a variety of bulk flow patterns. These range from simple attainment of gravitational equilibrium by 'preloaded' components that have been released from mechanical constraint, to more unusual effects such as interdigitated convective 'microfingers' (Saffman and Taylor, 1958; Preston *et al.* 1980; Davis and Acrivos, 1985; Nittmann and Stanley, 1986). Like the other generic processes outlined above, convective fingering effects may be embodied in morphogenetic and patterning mechanisms.

Finally, we note that many of the generic processes considered below exhibit *nonlinear* responses to relevant control variables. That is, small changes in interfacial tension, density, biosynthetic or diffusion rates, or even size and shape of a tissue domain, can lead to profound changes in the resulting morphology. This clearly bears on the succession of pattern and form during development. As we shall see, it also has implications for the evolution of phylogenetic lineages.

In what follows, we will attempt to situate generic mechanisms within the framework of existing developmental studies. The major generic effects considered in this review, their driving forces and their outcomes, are summarized in Table 1.

Phase separation and viscoelastic behaviors of embryonic tissues

Steinberg and his coworkers (Steinberg, 1962; Phillips and Steinberg, 1978; Steinberg and Poole, 1982) have considered the morphogenetic behavior of tissues in terms of their resemblance to liquids. Tissue fragments can flow in response to external forces, round up when suspended in a fluid medium, and coalesce with other such fragments, much like liquid droplets. Mixtures of cells from different types of tissue will sort out into homotypic 'islands' and 'lakes', and will eventually separate out completely, like a suspension of oil in water (Steinberg and Poole, 1982). After sorting out has occurred the relative configurations of the tissues are what would be predicted if tissues, like simple liquids, exhibited interfacial tensions with respect to their surroundings. In the case of tissues these surroundings could be other tissues, culture media, or artificial substrata (Fig. 1).

In terms of the categories and definitions of the previous sections, Steinberg's analysis asserts that many morphogenetic movements of tissues are explicable on the basis of the progression to equilibrium of closed, multiphase liquid systems. However, it is clear that living systems, which have both nutritional and waste removal requirements, cannot be truly closed, and that different tissue types are not uniform physical phases in the sense of the definitions above. Furthermore, cells and the tissues they constitute can actively generate motile and contractile forces, unlike liquids which flow passively. Finally, the molecular complexity of adhesive interactions between similar and dissimilar cell types (McClay and Ettensohn, 1987) seems difficult to reconcile with the explanation of equilibrium tissue configurations on the basis of a single quantitative scale of surface tension. In what sense, therefore, is there validity to Steinberg's application of the generic physical mechanism of equilibrium phase separation of immiscible liquids to tissue morphogenesis?

First, the liquid nature of certain tissues is an inevitable consequence of the fact that cells can slip past one another (Phillips *et al.* 1977) and dissipate strains that would otherwise cause bending or warping, changes in form that are characteristic of solids. The slippage may

Table 1. *Generic morphogenetic and patterning mechanisms considered in this review*

Mechanism	Driving force	Tissue feature acted upon	Outcome	References
Phase separation; wetting	interfacial tension	adhesive differences; viscosity	tissue spreading & engulfment; cell sorting out	Torza & Mason (1969)[1]; Newman *et al.* (1985)[1]; Forgacs *et al.* (1989)[1]; Steinberg (1978)[2]; Armstrong (1989)[2]; Frenz *et al.* (1989*a*,*b*)[2]
		elasticity	folding & distension of epithelial sheets	Mittenthal & Mazo (1983)[1,2]
Marangoni effect	interfacial tension	local variations in adhesion along common interface	interdigitating microfingers	Scriven & Sternling (1960)[1]; Napolitano (1984)[1]; Conklin (1905)[2]; Hörstadius & Sellman (1946)[2]
Sedimentation and buoyancy	gravity	density differences	ooplasmic segregation & rearrangement	Ancel & Vintemberger (1948)[2]; Malacinski (1984)[2]; Neff *et al.* (1984)[2]
Convective flows	gravity	local variations in density	interdigitating microfingers	Preston *et al.* (1980)[1]; Comper *et al.* (1987*a*,*b*)[1]; Conklin (1905)[2]; Hörstadius & Sellman (1946)[2]
Reaction–diffusion coupling	chemical potential, positive feedback	biosynthesis; tissue permeability	chemical waves	Turing (1952)[1,2]; Gierer (1981)[1,2]; Meinhardt (1982)[1,2]; Newman & Frisch (1979)[1,2]; Newman *et al.* (1988)[1,2]; Meinhardt (1988)[1,2]

[1] Physical mechanisms.
[2] Possible biological roles.

be purely passive, as with the molecular subunits of a non-living liquid, or can be facilitated by random motility of the living cells. Because of such internal rearrangements many tissues will not bend under application of force, or bounce back when it is removed: they will flow instead.

Second, subunits (molecules or cells) of a given liquid have characteristic binding interactions with one another despite their relative mobility. While there is no *a priori* physical reason why subunits in an arbitrary liquid should adhere to subunits of the same kind in preference to subunits from another liquid, it is a well-recognized fact of tissue biology that cells generally adhere to their own type in preference to other cell types (Townes and Holtfreter, 1955). This feature places tissues in a restricted category of liquids: they will generally be immiscible with one another. That is, when intermixed, tissues or their constituent cells will separate into distinct phases, and when confronted with one another, they will undergo characteristic spreading behaviors, not simply in analogy to oil and water, but for the same thermodynamic reasons. Inert particles can also be transported through tissues (Wiseman, 1977; Frenz *et al.* 1989*a*), most likely by adhesion-driven processes similar to those responsible for heterotypic cell sorting. Such particles can be used as probes of the adhesive environment in morphogenetically active tissues.

It should be noted that the arguments presented above hold whether homotypic adhesive preference of cells is achieved by *quantitative* differences in the strength of a single molecular adhesive mechanism, or by the more biologically plausible use of *qualitatively different* adhesion molecules by the two cell types. This is precisely because, in considering interactions between different types of cells, adhesive preferences based on qualitative differences in binding mechanisms can be ordered uniquely on a scale of greater/lesser binding strength *as if* the differences were simply quantitative.

Any two immiscible liquids will exhibit a characteristic interfacial tension when brought into proximity. Because a change in the area of the interface has a cost in terms of free energy, for a system at equilibrium, where the total free energy is at a minimum value consistent with all internal and external constraints, the interface will have a well-defined shape. In other words, the degree of mutual spreading of contiguous liquids, or engulfment of one liquid (or tissue) by another, will depend on the relative strength of the interfacial tension between the two liquids compared with the tensions at the interfaces of the two liquids with their other bounding substrata (Torza and Mason, 1969) (Fig. 1A,B). Because the relative balance of adhesive interactions at the various interfaces determines the extent of spreading, adhesion or deadhesion of a cell

population to different bounding surfaces can drive its concerted translocation in new directions (Forgacs *et al.* 1989).

Individual cells also exhibit fluid properties, and can locomote along adhesive substrata by an interfacial tension-driven process. This phenomenon has been termed 'haptotaxis' by Carter (1967). However, a single cell can only progress along its substratum by haptotaxis insofar as the substratum becomes progressively more adhesive over distance, *i.e.* if there is an adhesive *gradient*. In contrast, the concerted movement of populations of cells can be accounted for by the liquid-like spreading of the tissue as a whole along a substratum. This substratum must be adhesive, but need not contain an adhesive gradient. The apparent dependence of unidirectional migration of neural crest cells on intercellular contact (Newgreen *et al.* 1982) has been interpreted in terms of contact inhibition of movement (Newgreen and Erickson, 1986). But it may equally well reflect the propensity of such cells to interact with their adhesive environments as parts of coherent fluid tissues.

The spreading of the chick blastoderm on the inner surface of the vitelline membrane (Downie, 1976), the

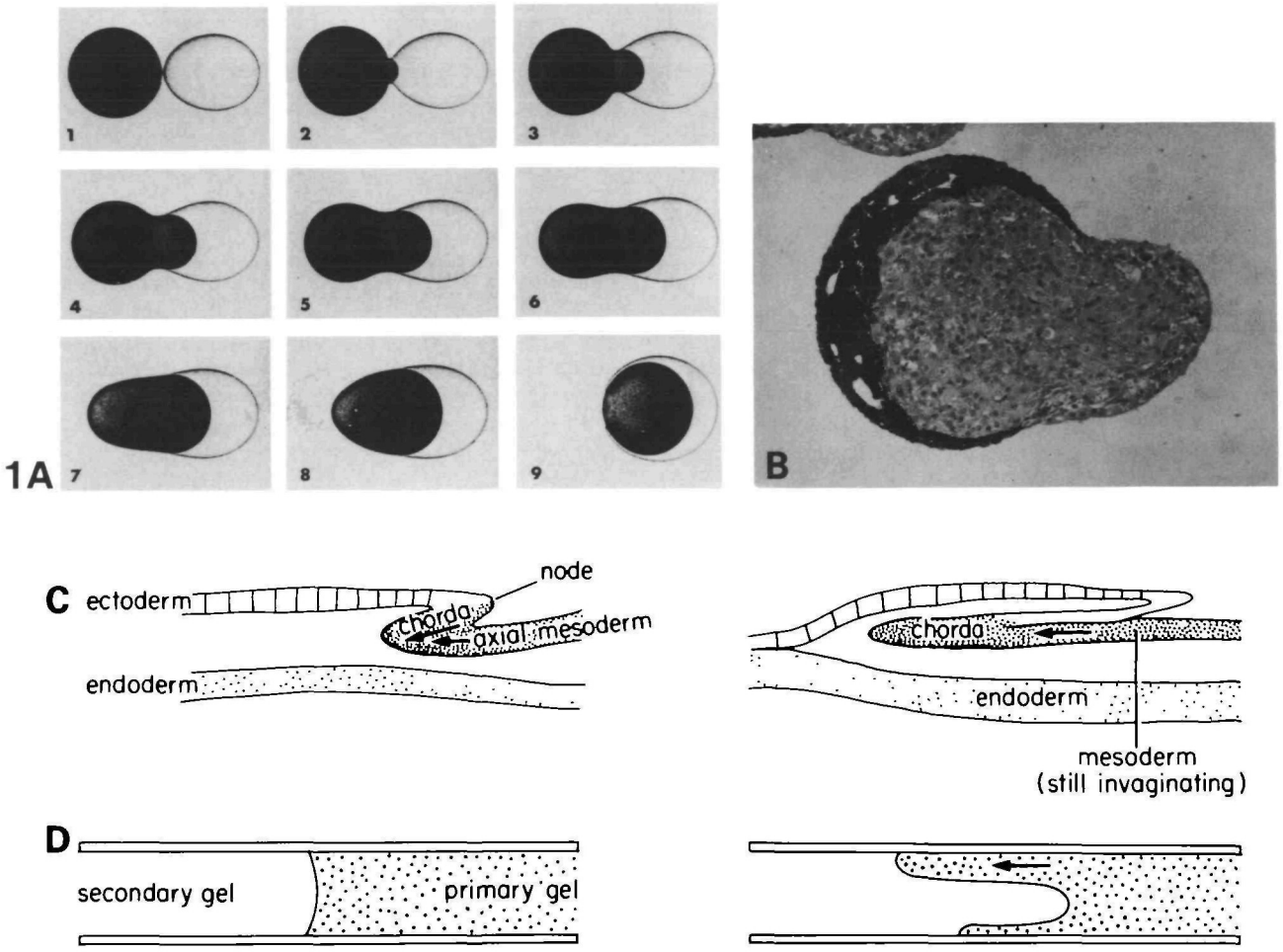

Fig. 1. Interfacial tension-driven effects in physical systems and possible roles in tissue interactions. (A) Photographs of stages of complete engulfing of water containing 1 percent malachite green (black drop) by a drop of polyglycol oil. Both drops were suspended in silicone oil. Frames 1 to 6 show the penetration of the aqueous phase into the oil phase, and frames 7 to 9 show the subsequent relaxation of the deformed water drop into the oil drop. Total elapsed time was 0.9 s From Torza and Mason (1969). Copyright 1969 by the AAAS. Picture courtesy of Dr T. G. M. van de Ven. (B) Spreading of 10-day-old chick embryo pigmented retinal tissue over the surface of an aggregate of 10-day-old embryonic heart tissue. Approximately spherical aggregates of the two tissues were placed in contact in hanging drop culture until they were firmly in adhesion, after which the composite aggregate was maintained in organ culture for 2 days. During this time, the pigmented retinal tissue spread as a monolayer over the surface of the heart aggregate. From Armstrong (1989). Picture courtesy of Dr Peter B. Armstrong. (C) Stages in mammalian gastrulation viewed in median section. (Left) Axial mesoderm has begun entering the primitive pit, forming the notochordal process or chorda. (Right) Chorda advances beneath the ectoderm followed by additional mesoderm, which then spreads laterally (out of the plane of section). Redrawn from Deuchar, 1975. (D) Matrix-driven translocation experiment (Newman *et al.* 1985) performed between two polystyrene plates (Forgacs *et al.* 1989), viewed from the side. Primary gel consists of cells or polystyrene beads suspended in type I collagen. Secondary gel contains 12.5 $\mu g\,ml^{-1}$ fibronectin in type I collagen. Spreading of primary gel along interfaces between secondary gel and upper and lower substrata occurs over approximately 5 min.

spreading of the chick embryonic epicardium over the myocardium (Ho and Shimada, 1978), gastrulation (Phillips and Davis, 1978) (Fig. 1C), teleost epiboly (Armstrong and Child, 1965), the elongation of the salamander pronephric duct over the lateral mesoderm (Poole and Steinberg, 1981), and the condensation of precartilage mesenchymal cells (Frenz *et al.* 1989*b*) are only some of the apparently goal-directed processes in the embryo likely to be driven, at least in part, by interfacial forces acting on liquid tissues.

Of course, tissues will not exhibit purely viscous behavior. The potential development of nonuniform tensions within connective tissues as a result of cell action on surrounding matrix fibers is a feature attributable to their partly elastic nature. The morphogenetic consequences of this property have been studied in model tissues consisting of cells suspended in defined collagen matrices (Bell *et al.* 1979; Harris *et al.* 1981).

The formation of 'compartments' in the developing insect cuticle (Crick and Lawrence, 1975; García-Bellido *et al.* 1976) can be plausibly treated as the progressive establishment of immiscible domains in epithelial sheets. Epithelia are interesting materials in that their cells are capable of exchanging neighbors (Keller, 1978), making them fluid in a two-dimensional plane. With respect to motions out of the plane, however, epithelia can act as elastic sheets. Indeed major features of epithelial morphogenesis can be explained in terms of deformations of epithelial sheets arising from strains generated by interfacial tensions between adjacent tissue blocks (Mittenthal and Mazo, 1983).

Mesenchymal and epithelial tissue behaviors thought to be due to generic physical mechanisms are inevitable consequences of recognized properties of these tissues such as homotypic adhesive preference of cells, and viscosity and/or elasticity. It is reasonable to suppose that genetically specified molecular mechanisms have evolved to reinforce these inherent tendencies, and to limit or specify the conditions for their occurrence. The evolution of mechanisms indifferent to, or in opposition to these forces, while formally possible, would probably have occurred less frequently.

Phase separation and adhesion-promoted cell transport in model connective tissues: matrix-driven translocation

The potential contribution of interfacial tension-driven effects to tissue morphogenesis can be demonstrated persuasively in a cell-free extracellular matrix system in which cytoplasm-generated motile forces play no role. If cells or cell-sized polystyrene latex beads with heparin-like molecules on their surfaces are suspended in a solution of type I collagen, and a drop of this suspension is placed contiguous to a second collagen drop, a sharp interface can form between the two regions. For this to occur, both the collagen and the particles must be in a certain concentration range (Newman *et al.* 1985; Forgacs *et al.* 1989). If the adhesive glycoprotein fibronectin is present in the second droplet there is a concerted translocation of particles and surrounding matrix, resulting from the expansion of the interface between the droplets near both bounding surfaces (Fig. 1D; Fig. 2). This effect, termed 'matrix-driven translocation' highlights certain generic physical properties of extracellular matrices that could readily play a part in morphogenetic processes in living tissues.

One of these properties is the apparent 'phase constituting' effect of extracellular fibers (Forgacs *et al.* 1989). The relative movement of matrix subregions by matrix-driven translocation could not occur if the regions remained miscible; a true interface is required (Forgacs *et al.* 1989). Surprisingly, under the minimal conditions required for translocation, the cells or beads in the initially populated region are, on the average, located more than ten particle diameters apart. Previous accounts of tissue engulfment behavior attributed the immiscibility of tissues of different origin to the adhesive preference of at least one of the interacting cell types for cells of its own kind (Steinberg, 1978; Steinberg and Poole, 1982). In the matrix-driven translocation system (and in the mesenchymal tissues for which it serves as a physical model), a mechanism must exist to promote phase formation where cells are not directly in contact.

'Percolation theory' (Stauffer, 1985) is a mathematical tool for analyzing cluster formation in physical systems as varied as the gelling of polymers (Bouchaud *et al.* 1986), formation of galaxies (Schulman and Seiden, 1986) and the spreading of forest fires (MacKay and Jan, 1984). Using this approach it has been shown that the relative concentrations of cells and extracellular fibers in the matrix-driven translocation system, and in typical mesenchymal tissues, are sufficient to induce 'macroscopic clusters', or networks, in subregions of tissues. According to percolation theory, the formation of such a network causes phase separation of the region containing it from similar regions that do not. The resulting interface provides the conditions for coherent movement of populations of cells by interfacial tension-driven effects (Forgacs *et al.* 1989). These studies also suggest that tissue regions populated with cells and adjacent nonpopulated extracellular matrices can constitute distinct physical phases, a finding that is relevant to the analysis of gastrulation, neural crest migration, and other processes in which cell populations enter cell-free spaces (See Trelstad (1984) for reviews).

Another property of the matrix-driven translocation system that bears on mechanisms of morphogenesis in living systems is the demonstration that the spontaneous spreading behavior of tissues may be impeded by low energy hindrances at their interfaces, which can, in turn, be overcome by specific, but energetically weak, molecular interactions. The force for the concerted movement of cell or bead suspensions in the matrix-driven translocation system is related to the reduction in free energy brought about by spreading of one collagen phase upon the other. This spreading can occur in the absence of fibronectin at collagen concentrations slightly below that required for the fibronectin-

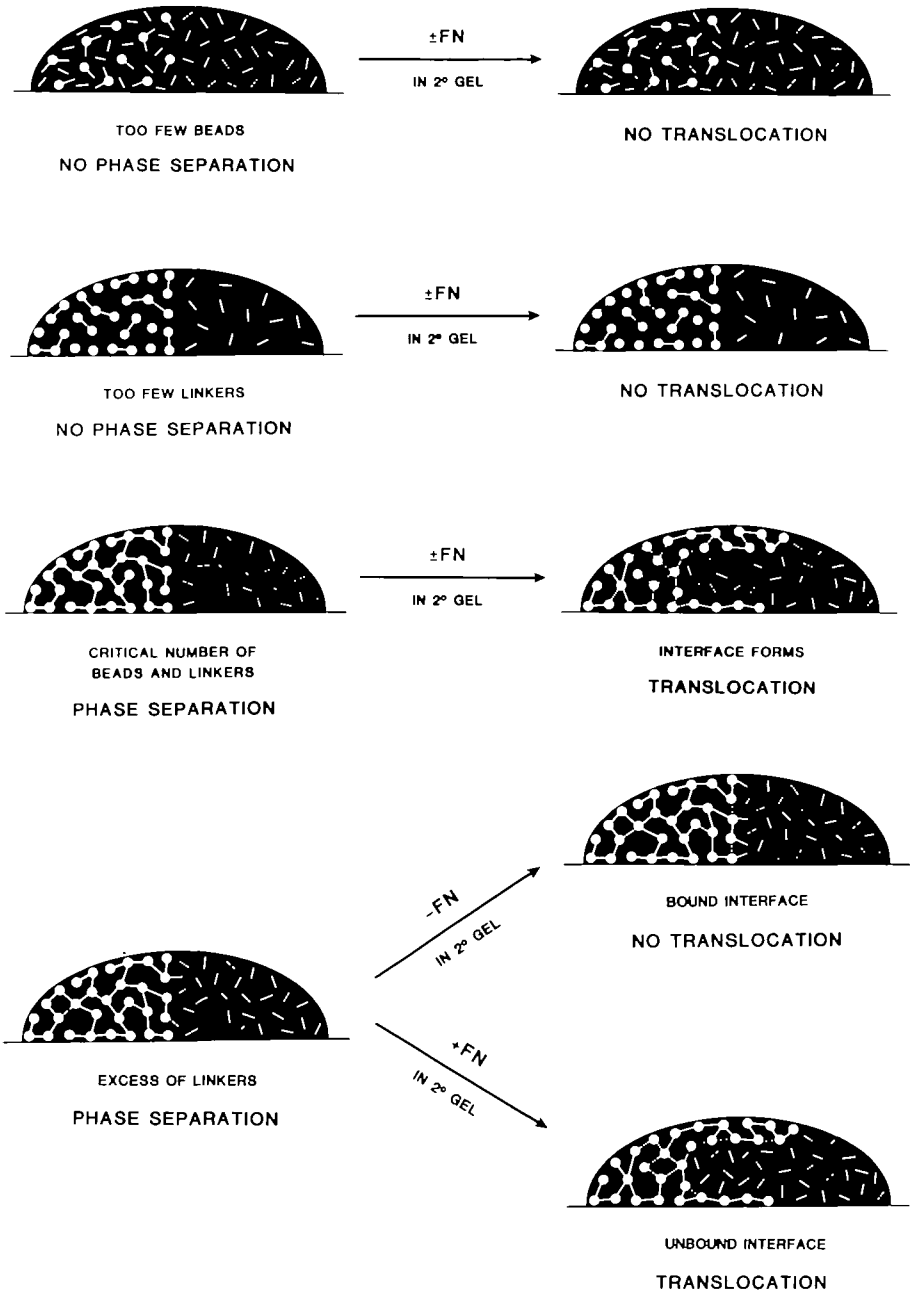

Fig. 2. Interpretation of formation of separate tissue phases by generation of networks. Cells or polystyrene particles with appropriate surface characteristics ('beads') are mixed with soluble collagen and placed next to a drop of collagen which contains no particles, but may or may not contain a substance adhesive to the cell or bead surface (fibronectin: 'FN'). When too few particles or too few collagen fibers ('linkers') are present, no pervasive network forms, and no phase separation takes place. When a critical number of particles and linkers are present network formation and phase separation occurs, and one phase may tend to spread upon or engulf the other. At higher collagen concentrations spreading may be impeded and require adhesive interactions at the interface to overcome the hindrance. From Forgacs *et al.* (1989).

dependent effect (Forgacs *et al.* 1989; Fig. 2). At the higher collagen concentration this spontaneous spreading is hindered (possibly by excess binding of particles to matrix at the interface), but can be released by the weak, but highly specific interaction of the cell or bead surfaces with the amino-terminal heparin-binding domain of fibronectin (Newman *et al.* 1987; Khan *et al.* 1988; 1990).

In complex cell–matrix systems, where there are multiple adhesive interactions, the balance of forces can potentially be tipped by weak interactions. This provides a likely locus for the intersection of generic and genetic mechanisms. In the model system described, for example, the generic phenomenon of the mutual spreading of two liquids provides the driving force for a morphogenetic event that can be hindered or released by weak but highly specific biomolecular interactions at the interface.

Gravity and cytoplasmic reorganization in the amphibian egg

Another illuminating example of the potential relationship between generic and genetic mechanisms in morphogenesis is provided by the amphibian egg. Eggs of anuran species have a thin, outer cortical layer of cytoplasm that is immiscible with the central egg cytoplasm. Following fertilization in anurans there is a 30° rotation of this cytoplasm relative to the deeper cytoplasm (Ancel and Vintemberger, 1948; Vincent *et al.* 1986; Fig. 3). In some species this rotation has the

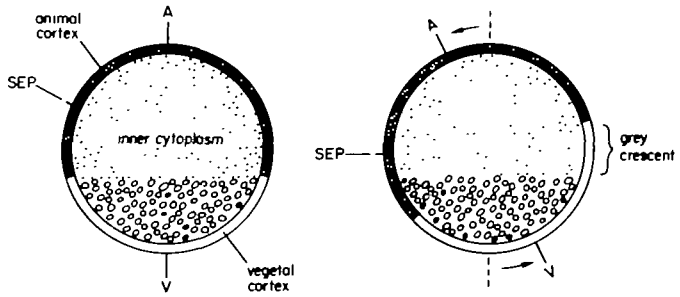

Fig. 3. The cortical/cytoplasmic rotation in anuran embryos. A 30° rotation of the cortex relative to the inner cytoplasm is required for normal dorsoventral polarity to be established. Diagrammatic sections are shown before (left) and after (right) rotation. The cortex rotates so that the sperm entry point (SEP) moves vegetally. The grey crescent visible in *Rana pipiens* embryos is formed by the overlapping of pigmented animal pole cytoplasm by nonpigmented vegetal pole cytoplasm. Redrawn from Elinson and Rowning (1988).

effect of revealing a lightly pigmented region of deep cytoplasm on one side of the egg, the grey crescent. This region, or its equivalent, marks the future dorsal area of the embryo.

Inhibition of the cortical cytoplasmic rotation by UV, cold shock, or chemical means severely perturbs dorsoventral axis formation in the embryo. In partially inhibited eggs, the amount of rotation is correlated with the extent of axis formation (Vincent and Gerhart, 1987). Moreover, in inverted eggs, the direction of shift of major cytoplasmic components determines the dorsoventral polarity of the ensuing embryo (Gerhart *et al.* 1981; Neff *et al.* 1984). These experiments have provided persuasive evidence that subcortical rotation is a necessary early step in embryonic axis specification.

Ancel and Vintemberger (1948) suggested that the relatively rigid egg cortex, upon mechanical release from the underlying cytoplasmic core midway during the first cell cycle, slips to one side by 30° under the influence of gravity, in normally oriented eggs. The twinning effects of unit gravity on experimentally rotated eggs (Gerhart *et al.* 1981), and the ability of unit gravity to effect a proper overlap between cortical and deep cytoplasm in eggs whose normal rotation is inhibited by UV (Neff *et al.* 1984; Vincent & Gerhart, 1987), are consistent with a role for gravity in normal axis specification.

However, a mechanism of axis specification driven exclusively by gravity is contradicted by results of Vincent *et al.* (1986), who embedded *Xenopus* eggs in gelatin so that the cortex could not move, and found that the cytoplasmic core, including the denser vegetal regions, rotated up one side of the cortex by 30°, working against gravity. Clearly the egg must also have a means other than gravity to drive the rotation.

The presence of an oriented array of microtubules in the shear plane between the cortex and subcortical cytoplasm (Elinson and Rowning, 1988) suggests that the force-generating mechanism might be similar to the energy-consuming microtubule-dependent organelle transport systems found in other cell types (Vale, 1987). But because gravity may suffice to drive cytoplasmic reorganization under all but the most unusual circumstances, it could have been the phylogenetically original determinant of cortical rotation and axis specification. A specific microtubule-based force-generating mechanism may have subsequently been selected on the basis of its ability to enhance the dependability of an event originally driven by a generic physical process.

Convective mechanisms

Concerted relative motion of different regions of a fluid is known as *convection* (Velarde and Normand, 1980). If the different fluid regions have an interfacial tension between them, convective flows can take place by the progression to adhesive equilibrium from a metastable state, as proposed for the matrix-driven translocation system above. Flows can also result from instabilities in the contour of the interface due to local variations in interfacial tension. These variations can be brought about, in turn, by local changes in composition along the interface. Flows resulting from variations in the interfacial tension are examples of Marangoni convection (Scriven and Sternling, 1960). Both classes of phenomena depend on generic mechanisms that can occur in living tissues.

Even in the absence of interfacial tension, different parcels of a fluid may undergo convection if they differ in density or viscosity, and a force that can act on these differences is present. Regions of different density of a heterogeneous cytoplasm, tissue or extracellular matrix, if they are out of gravitational equilibrium, are clearly subject to morphogenetic rearrangement by gravity as indicated in the previous section. Contiguous cytoplasmic compartments, tissues or matrices that differ in viscosity (a measure of internal frictional forces relative to flow velocity) will flow relative to one another if subjected to external pressure.

Convection in physical systems is commonly brought about by temperature gradients, which would not typically occur in developing tissues. But the proximate causes of thermal convection, as in some of the examples discussed above, are actually density or surface tension inhomogeneities, which, at least in tissues, can result from metabolic or biosynthetic processes rather than heating.

A number of developing systems exhibit a microfinger morphology in which distinct cytoplasmic or tissue components interdigitate with one another. The width of each microfinger is usually on the order of tens to hundreds of μm. Some examples are shown in Fig. 4. These microfingers can be transient, like the residual streamers resulting from downward flow of yellow cytoplasm in the newly fertilized egg of the ascidian *Styela partita* (Conklin, 1905) (Fig. 4A), or in the migration pattern of endogenous and ectopic neural crest cells in the axolotl embryo (Hörstadius and Sellman, 1946) (Fig. 4B). They can also be stable, as in the fully developed pattern of kidney collecting tubules

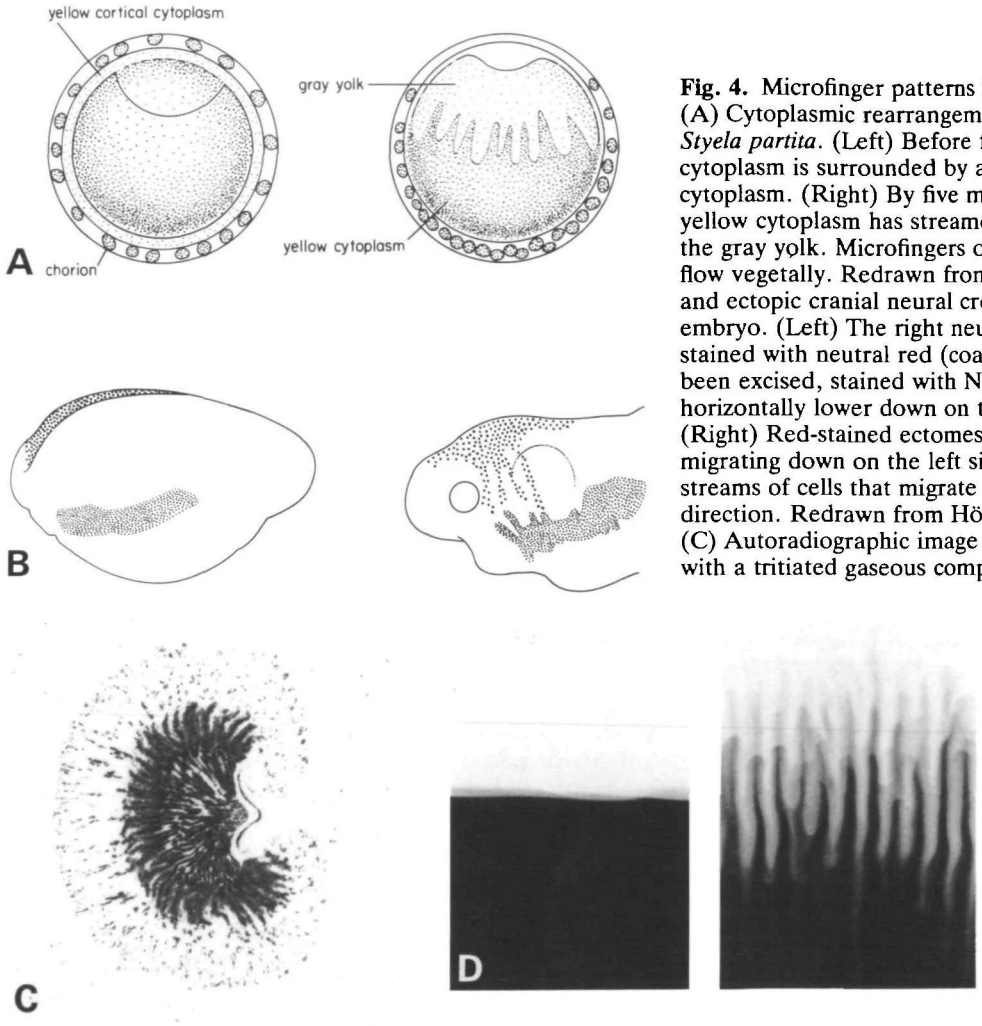

Fig. 4. Microfinger patterns in living and nonliving systems. (A) Cytoplasmic rearrangement in the egg of the tunicate *Styela partita*. (Left) Before fertilization, inner gray yolky cytoplasm is surrounded by a peripheral layer of yellow cytoplasm. (Right) By five minutes after fertilization the yellow cytoplasm has streamed to the vegetal pole, exposing the gray yolk. Microfingers of yellow cytoplasm continue to flow vegetally. Redrawn from Conklin (1905). (B) Normal and ectopic cranial neural crest migration in the axolotl embryo. (Left) The right neural ridge of the head has been stained with neutral red (coarse stippling). The left ridge has been excised, stained with Nile blue and implanted horizontally lower down on the same side (fine stippling). (Right) Red-stained ectomesoderm from the right side is migrating down on the left side, as it does normally, meeting streams of cells that migrate from the graft in the dorsal direction. Redrawn from Hörstadius and Sellman, 1946. (C) Autoradiographic image of a slice of rat kidney perfused with a tritiated gaseous compound. The filled collecting ducts, which are between 30 μm and 50 μm wide, are evident in a microfinger arrangement. From Charpak *et al.* (1989). Picture courtesy of Dr G. Charpak. (D) Time evolution of structured flows in a polymer system containing dextran and poly(vinyl-pyrrolidone) (PVP). The PVP in the lower layer was coupled to a blue dye. (Left) Initial preparation; (Right) After 40 min. The microfingers are on the order of 500 μm in width. Adapted, with changes, from Comper *et al.* (1987*a*).

(Fig. 4C). These microfingers suggest the action of convective mechanisms, which, in multicomponent fluids with or without interfacial tension (Saffman and Taylor, 1958; Nittmann and Stanley, 1986) or in settling particle suspensions (Davis and Acrivos, 1985), can give rise to microfingers on the scale seen in these living systems.

Suspensions of cells or inert particles can spontaneously organize into countercurrent microfinger patterns under the influence of gravity. Countercurrent streaming associated with random density fluctuations has been observed in *Tetrahymena* cultures (Winet and Jahn, 1972). Microfinger morphologies have also been observed during sedimentation of polydisperse and binary particle suspensions at high solid concentration (reviewed in Davis and Acrivos, 1985). Whitmore (1955) performed experiments with suspensions containing a mixture of heavy particles and neutrally buoyant particles. The initially homogeneous suspension quickly organized into a stratified system with microfingers moving in the vertical plane: the buoyant particles (~100 μm in diameter) were carried in the upward moving fingers and the heavier particles in the downward moving fingers. More recently, Weiland and McPherson (1979) found that after adding buoyant particles to an otherwise uniformly settling suspension, there was a rapid lateral segregation of the two species of particles into countercurrent vertical fingers. The mechanisms for these gravity-driven convective patterns involving large particles is not known, but clearly they may be relevant to living systems with a heteropycnic population of cells that can be made to flow relative to one another.

Remarkably, gravity-driven convective microfingers can even occur in spatially nonuniform multicomponent fluid systems that are initially in gravitational equilibrium, i.e. where the denser fluid is layered below the less dense fluid. This latter effect is a general property of polymer solutions, and has been demonstrated for proteoglycans (Harper *et al.* 1984), collagen undergoing fibrillogenesis (Ghosh and Comper, 1988), and substrate and product gradients in the presence of a converting enzyme (Comper and Preston, 1981), and predicted to occur for the assembly–disassembly of microtubules in axons (Comper *et al.* 1983). The origin of the effect resides in the fact that relaxation of concentration gradients in multicomponent systems is often accompanied by the formation of 'microdensity inversion' (Comper *et al.* 1987*b*). This is an unstable state in which a layer of denser fluid transiently overlays

a thin layer of less dense fluid. The heavy layer tends to sink and the lighter layer to float in striated countercurrent flows. This dynamic stratification is maintained by a constant exchange of material between flows, and by gravity acting on density gradients at the interface of oppositely moving flows. In an unrestrained system these flows grow in the vertical plane (Fig. 4D).

Convective microfingers or flows can passively transport inert particles or living cells at a rapid rate (i.e. with velocities of mm per day to mm per min). Such flows are able to move through 90° and 180° bends in capillary tubing. In these cases, the upward moving flow remains uppermost to the surface of the tubing with respect to the downward moving flow. The flows may rotate with respect to one another in attaining these positions.

Whereas gravity provides the driving force in the experimental systems described, it causes transport in a direction counter to what would be expected on the basis of initial density distribution. Moreover, it can exert its effect with only a minimal gravitational gradient. Indeed, microfinger formation and rapid transport of cells can occur in systems displaced as little as 4° from the horizontal (Comper *et al.* 1987*a*). This makes a morphogenetic role for these processes reasonable, since the probability is very low that any channel or mechanically constrained space in a developing organism would remain perfectly horizontal. In addition, the dependence of the *rate* of flow on the magnitude of the gravitational field is very weak: the measured rate of transport in one such system was proportional to $g^{0.2}$ (Preston *et al.* 1983); a flow rate proportional to $g^{0.33}$ has been predicted on theoretical grounds for a general class of convective systems (Turner, 1973). Consequently, experimentally neutralizing or attenuating the effect of gravity-driven flows by changing the orientation of the system may be difficult because of the low exponent of gravity. One might falsely conclude that gravity is not essential to transport if the rate of flow were insensitive to these manipulations.

The possibility that gravity-driven convection can occur in the extracellular matrices of developing embryos and provide guidance for cell translocation has been considered (Comper *et al.* 1987*a*). This may be relevant to the microfinger morphology described for neural crest migration by Hörstadius and Sellman (1946) (Fig. 4B), although the possibility that the invading sheet of neural crest cells is divided into streams by anatomical obstructions (Noden, 1988) must certainly also be considered. It is interesting, however, that ventrally transplanted ectopic neural crest cells also break into microfingers of similar dimension in a region of the embryo distant from the endogeneous streams (Hörstadius and Sellman, 1946; Fig. 4B). This parallels the countercurrent aspects of the convective processes. The possibility of convective flows in the extracellular matrix may also be relevant to studies by Bronner-Fraser (1982), who demonstrated that nonmotile cells or latex beads were translocated along neural crest pathways into which they had been implanted. The translocation of inert particles was highly selective with respect to particle surface characteristics in Bronner-Fraser's experiments, a property not exhibited by matrix flows driven purely by gravity. However, gravity-driven convection may act in a cooperative fashion with matrix-driven translocation (see previous section). In this case, interfacial interactions between oppositely moving flows would ensure that cells or particles with different surface characteristics are translocated selectively.

Undoubtedly cells also use their ability to locomote independently of one another in translocating through the embryo (Newgreen and Erickson, 1986). But the possible contribution to cell translocation of generic effects such as gravity-driven and interfacially-driven convection within the extracellular matrix may help explain the microfinger morphology of the collective movement of these cells, as well as aspects of the movement that occur independently of intrinsic cell motility.

Reaction–diffusion mechanisms

Diffusible substances can elicit various responses from cells, and can thereby act as determinants of morphogenesis or pattern formation ('morphogens'). Without active processes to maintain them, gradients* of diffusible substances will dissipate over relatively short time intervals in regions of the size of embryonic fields; therefore sources and sinks of the relevant agents or their precursors or metabolites are minimally required for spatial patterns that are *stationary* (i.e. unchanging with time). In contrast to convection, which leads to change in the relative positions of physically distinct cytoplasmic, tissue or extracellular matrix components, chemical non-uniformity does not by itself constitute a morphogenetic outcome. Generic processes that can reasonably lead to chemical gradients in tissues are described below, but the morphogenetic and patterning roles of these gradients are only manifested when cells respond to local concentrations of the relevant substances by undergoing mechanochemical or differentiative changes that are, by and large, 'genetic.'

Localized or monotonically distributed sources or sinks of diffusible morphogens will readily lead to stationary monotonic gradients across a tissue domain or field (Crick, 1970). Less obvious was the discovery by Turing (1952) that feedback interactions between reacting and diffusing components could lead to complex stationary gradients of morphogens that could mimic, and perhaps provide the basis for, periodic embryonic structures.

These patterns depend on the consumption of free energy to maintain themselves as spatially nonuniform, but temporally constant, entities. Along with stable *convective* patterns (which also can be maintained by

*In common biological usage 'gradient' is taken to mean a monotonic distribution of a substance. Here we use the term in the more inclusive physical sense of any nonuniform distribution, regardless of shape.

the expenditure of energy), stationary chemical nonuniformities produced by reaction–diffusion coupling in open systems have been generalized into a theory of 'dissipative structures' by Prigogine, Nicolis and their coworkers (Prigogine and Nicolis, 1967; Nicolis and Prigogine, 1977; Prigogine, 1980). The mechanism for establishment of these unusual states in reaction–diffusion systems can be understood by a graphical analysis first presented by Maynard Smith (1968) (Fig. 5A–E).

We will make the following assumptions:

(i) two substances, A and B, which influence one another's synthesis and consumption, are produced throughout a row of cells.

(ii) there is a balance of the rates of transport of precursors and metabolites of A and B, and the rates of synthesis and consumption of A and B, such that both substances are at steady-state concentrations within the row of cells.

For most biochemically feasible interactions between A and B, the steady-state concentrations of these molecules will not vary along the row of cells, even if one or both of them can diffuse (Fig. 5A). However, under special circumstances a different steady-state can be attained, in which the concentrations of A and B change from point to point within the tissue. To see how this may occur, consider some additional assumptions:

(iii) A has a positive effect on the synthesis of both A and B

(iv) B has an inhibitory effect on the synthesis of A

(v) B diffuses faster than A

The consequence of these assumptions is that if the concentration of A undergoes a small local fluctuation to a value above its uniform steady-state level (Fig. 5B) it will cause additional A and B to be formed (Fig. 5C). Because B diffuses out faster than A, at some distance away from the initial peak B will be inhibiting the synthesis of A in cells in which A is at its original uniform steady-state level (arrow, Fig. 5C). At this point A will decline below that concentration (Fig. 5D), causing the concentration of B also to drop (Fig. 5E).

It can be seen that this process will lead to a series of peaks and valleys in the concentrations of both A and B. In some systems a nonuniform distribution of morphogens will be attained in which production and breakdown of each component will balance at every point along the tissue, so that the final distribution, although *spatially* nonuniform, will be unchanging with time, i.e. it will constitute a new steady-state. The number of peaks and valleys that will be in place when the system finally reaches this steady-state will depend on reaction and diffusion rates, the size and shape of the spatial domain in which these events are occurring, and the modes of utilization of A and B at the boundaries of the domain.

The example analyzed above is only one of several generic reaction–diffusion mechanisms that can give rise to periodic patterns (Turing, 1952; Prigogine and Nicolis, 1967; Nicolis and Prigogine, 1977). A feature common to virtually all of them is the presence of a self-enhancing or 'autocatalytic' property of one of the components, and inhibition of the less mobile component by the more mobile one (Gierer, 1981; Meinhardt, 1982). Although reaction–diffusion processes demonstrably lead to the formation of intricate, spatially nonuniform patterns in nonliving chemical systems (Ross *et al.* 1988; Castets *et al.* 1990), biosynthetic pathways are typically self-limiting rather than self-enhancing, a fact that has restricted the serious consideration of such processes as feasible developmental mechanisms. This reservation has now been removed with the discovery of self-enhancement of the biosynthesis of certain soluble growth and differentiation factors, such as transforming growth factor beta (TGF-β) (Van Obberghen- Schilling *et al.* 1988), and of some gene regulatory proteins, such as *c-jun* (Angel *et al.* 1988).

In developing systems self-enhancement and cross-inhibition of key factors may be direct, but they may also be indirect. Despite this potential complexity, testable hypotheses can be framed for biological pattern formation based on reaction–diffusion processes. This is because many predictable properties of such systems, such as symmetries of allowable patterns, dependence of pattern on reaction and diffusion rates of key components, and changes in steady-state patterns resulting from changes in tissue size and shape, can be relatively robust with respect to variations in underlying biochemistry (Newman and Frisch, 1979; Newman *et al.* 1988).

Some periodic patterns that form during embryogenesis of insects and vertebrates, and which have been suggested to be due, in part, to reaction–diffusion patterning mechanisms, are shown in Fig. 5F and G. The striped pattern of expression in *Drosophila* embryos of the primary pair-rule genes (the initial pattern of the *even-skipped (eve)* protein product is shown) depends on the prior nonuniform distribution of gap gene products, none of which exhibits spatial periodicity at this stage (Jäckle *et al.* 1986). Meinhardt (1988) has presented a model for the patterned expression of the pair-rule genes, which assumes that these genes specify components of reaction–diffusion pattern-forming systems. A pair-rule gene is presumed to be activated at the borders of regions of expression of a gap gene; autocatalysis and lateral inhibition in the hypothesized pair-rule system ensures that stripes will form, but only if the autocatalysis saturates at low concentrations of pair-rule gene product. The formation of patches, rather than stripes, of pair-rule product is sometimes seen in embryos carrying a mutation of a pair-rule gene. This morphology is predicted from purely dynamical considerations when saturation of autocatalysis is not achieved (Meinhardt, 1988).

In contrast to this proposed account of the striped pattern of pair-rule gene expression are findings by several groups that these genes contain promoter elements that bind to unique combinations of gap gene proteins (reviewed in Akam, 1989). For example, the protein products of the gap genes *hunchback* and *Krüppel* bind to specific sites in the *eve* promoter (Štanojević *et al.* 1989), and deletion of these sites leads

to loss of specific stripes (Goto *et al.* 1989). The implication of these studies is that each pair-rule stripe is uniquely induced by a set of specific instructions, and that dynamical pattern-generating systems are not required for pattern formation (Akam, 1989).

If the possibility of an interplay between generic and genetic mechanisms for the striped distribution of pair-rule gene products is considered, they may be seen as playing mutually reinforcing roles. One suggestion is that feedback circuits may amplify or sharpen discontinuities and boundaries originally specified by genetic 'specific instruction' mechanisms (Akam, 1989). Alternatively, the generic physical processes may actually be the primary ones (at least in evolutionary terms). With the 'rough' pattern specified by a dynamical reaction–diffusion mechanism, molecular evolution would have had a globally organized morphological substrate which it could stabilize and refine over time.

A second example of possible interactions between genetic and reaction–diffusion mechanisms is provided by the development of the vertebrate limb (Newman, 1988*a*) (Fig. 5G). Here the periodic pattern is generated sequentially, with the number of 'stripes' (corresponding to parallel skeletal elements) increasing in a discontinuous fashion over time. This pattern could be generated by a mechanism like that schematized in Fig. 6 (Newman and Frisch, 1979). The morphogen is presumed to promote cellular aggregation or condensation, which precedes cartilage differentiation (Fell and Canti, 1934; Thorogood and Hinchliffe, 1975). It therefore provides a 'prepattern' for the skeleton. As is typical for reaction–diffusion systems, the number of morphogen peaks and valleys is sensitive to the size and shape of the reaction vessel, or tissue domain. In this model, the increase in peak number is tied to the *decrease* in proximo-distal length of the undifferentiated zone, which appears to occur in relatively abrupt steps (Summerbell, 1976). Each different-sized compartment will have a characteristic number of chemical waves when the system reaches a steady state. (An increase in anteroposterior length of this zone, as occurs in the human limb, would also increase the peak number in this model (Newman and Frisch, 1979)).

This dynamical pattern-forming mechanism may be embodied in molecules such as TGF-β (Massagué, 1987) which stimulates its own synthesis (Van Obber-

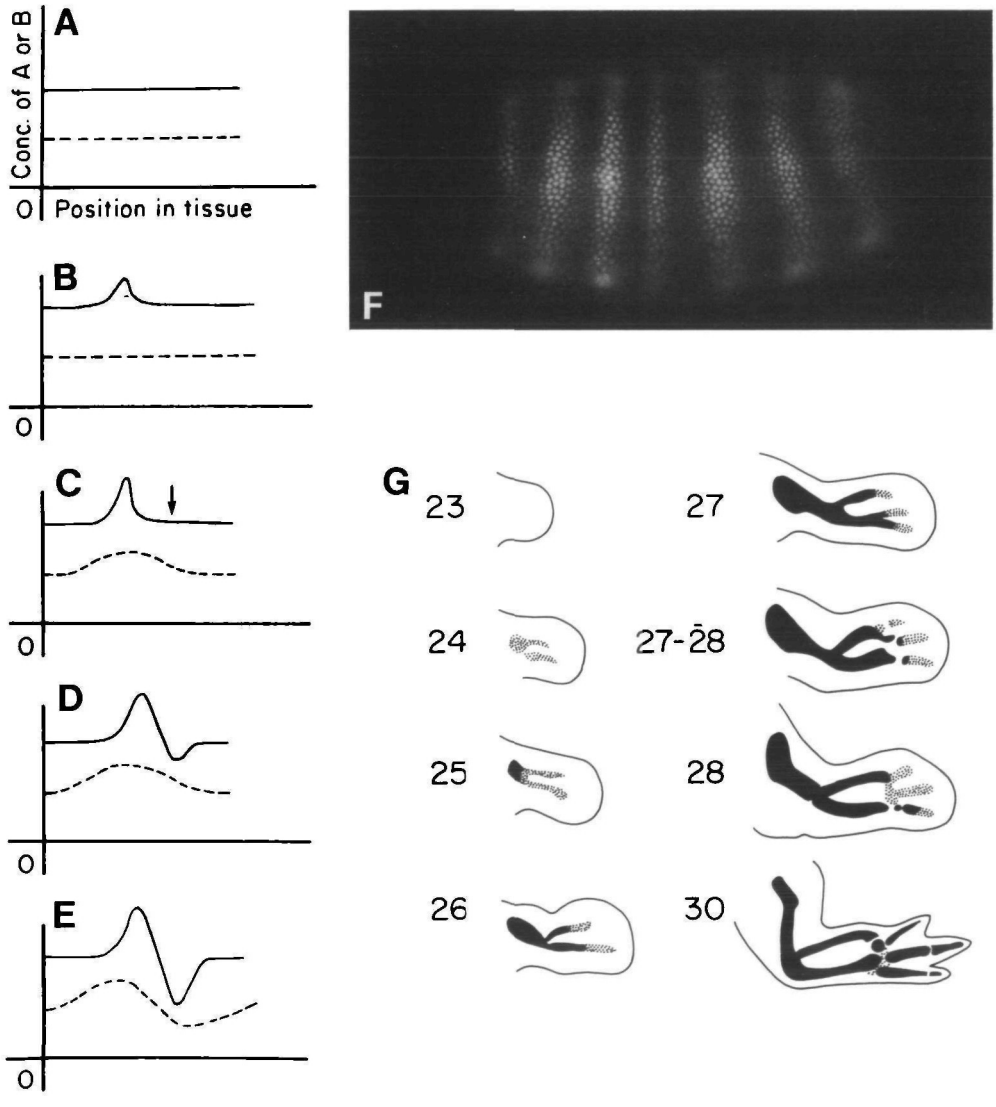

Fig. 5. Chemical wave generation by a reaction–diffusion mechanism, and examples of stripe patterns during development. (A–E) Graphical representation of chemical wave formation, based on Maynard Smith (1968). See text for discussion. (F) *Drosophila* blastoderm stage embryo showing early *even-skipped* protein pattern (white stripes). Picture courtesy of R. Warrior and M. Frasch. (G) Progress of chondrogenesis in the chick wing bud between 4 and 7 days of development. Solid black regions represent definitive cartilage; stippled areas represent early cartilage. Stages are those of Hamburger and Hamilton (1951). From Newman and Frisch (1979), copyright 1979 by the AAAS.

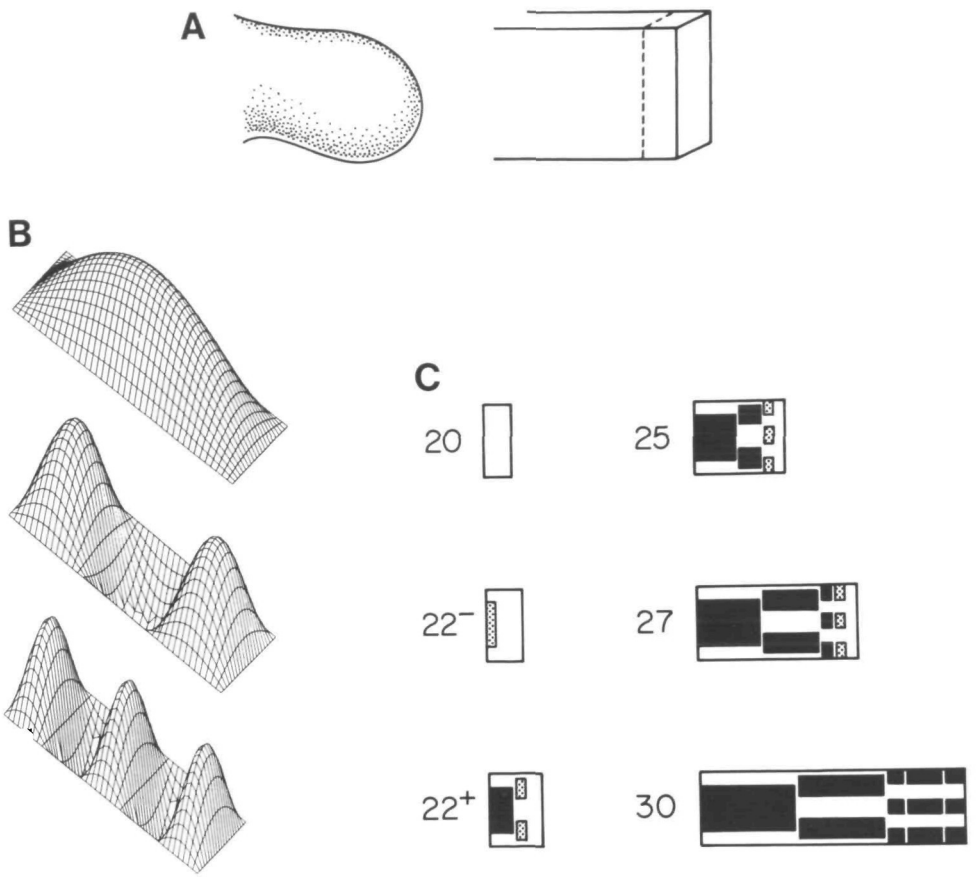

Fig. 6. Interpretation of chick limb development based on a reaction–diffusion mechanism. (A) (Left) Drawing of a 5-day wing bud. (Right) Schematic representation of 5-day wing bud with as yet unpatterned distal mesenchyme demarcated by dashed line. (B) Graphs representing predicted distribution of TGF-β and fibronectin in the prechondrogenic distal mesenchyme at approximate stages 21 (top), 23 (middle), and 25 (bottom) of development. (C) Predicted cartilage pattern based on schematic model. A and B adapted with changes from Newman and Frisch (1979); C from Newman and Frisch (1979), copyright 1979 by the AAAS.

ghen-Schiller *et al.* 1988), and which also stimulates the synthesis of the more slowly diffusing extracellular matrix molecule fibronectin (Ignotz and Massagué, 1986). Fibronectin, in turn, promotes precartilage cell condensation (Frenz *et al.* 1989*a*,*b*). Lateral inhibition of the positive feedback effects, which is required in order for the condensations to be spatially confined rather than encompassing the entire domain of competent tissue, may be the result of indirect effects.

While such reaction–diffusion mechanisms may regulate the development of a 'rough' skeletal pattern, they cannot readily account for refinements such as the anatomical distinctiveness of the radius and ulna, the different digits, or the skeletons of the fore and hind limbs as a whole. This probably requires the superimposition of 'genetic' modifiers such as homeobox-containing nuclear proteins (Gehring, 1987; Dollé *et al.* 1989*a*) or retinoic acid receptors (Maden *et al.* 1988; Dollé *et al.* 1989*b*). Homeobox proteins are nonuniformly distributed in developing limb buds (Oliver *et al.* 1988; Dollé *et al.* 1989*b*) and may refine the rough pattern by influencing local patterns of chondrogenic gene expression. Exogenously administered retinoids can change the number of skeletal elements and affect the symmetry of the pattern (Tickle *et al.* 1985). Endogenous retinoids (Thaller and Eichele, 1987) or their receptors may modify the pattern directly, by their effect on chondrogenesis, or indirectly, by their effect on tissue growth (Ide and Aono, 1988; Paulsen and Solursh, 1988).

Implications for evolution

The proposal that generic physical effects could be responsible for major structural and patterning changes during ontogeny immediately raises the question of the significance of these processes in setting phylogenetic trends. We have argued that machine-like molecular mechanisms, in contrast to generic physical effects, typically act locally rather than globally in spatial terms. Analogously, the incremental events by which genetic mechanisms evolve would most generally act 'locally' in phylogenetic terms, conserving existing successful body plans rather than causing species to undertake the first steps leading to major structural rearrangements, the adaptive significance of which would not generally be realized in intermediate forms.

The action of generic physical mechanisms in morphogenesis and pattern formation provides a way out of the conundrum of evolution through nonadaptive intermediates, for it suggests how, in systems embodying no more than the standard nucleic-acid-based mechanisms of inheritance, a genetically distant endpoint may be immediately brought 'into sight'. We envision the consequences of dominantly acting mutations that affect features of embryos and tissues susceptible to

generic physical mechanisms. As discussed above, these mechanisms often exhibit nonlinear responses to changes in control variables. Thus, a small change in density of an ooplasmic determinant could lead to large changes in its spatial distribution. A minor alteration in interfacial tension between two tissue compartments could strikingly change their relative configurations. A pre-existing biosynthetic circuit which contains a diffusible component could be thrown into a pattern-generating mode by a small change in the ratio of reaction and diffusion rates. In each of these cases, profound alterations in morphology, reproducible from generation to generation, would ensue, virtually at one stroke. If the resulting variants proved successful in establishing and populating new niches, eons of genetic evolution could follow, stabilizing and reinforcing the new outcome. The alternative model, i.e. major morphological evolution by increments, would be analogous to bridging a chasm of indeterminate breadth.

This scenario is consistent with the tempo of macroevolutionary change, which has been characterized as consisting of long periods of morphological stasis punctuated by episodes of rapid structural reorganization (Gould and Eldredge, 1978). It is also consistent with the redundancy of morphogenetic mechanisms, and the related 'overdetermination' of morphogenetic outcomes, that are well-recognized aspects of development. It should be noted that it has not been necessary to invoke the concept of a 'genetic program', or the change thereof, to account for ontogeny or phylogeny in this perspective (see Oyama (1985), and Newman (1988*b*) for critical discussions of this concept). Neither developmental 'routines' nor the evolutionary record need be inscribed in the genes if the participation of generic physical processes is taken into account.

Our perspective is complementary to the recognition that static physical or 'architectural' constraints can guide developmental outcomes (Horder and Martin, 1978; Springer and Mednick, 1985), or may give rise to epiphenomena that can be coopted to new functions during the course of evolution (Gould and Lewontin, 1979). But, by focusing on *dynamical* effects of a generic nature, we suggest a role for these phenomena beyond that of 'constraint'. In this interpretation, *genetic* mechanisms would often serve to limit or constrain pathways that have been set by generic physical effects, a reversal of the usual attribution of all morphological novelty to random genetic change.

Conclusions

We have described a number of effects that could lead to the rearrangement of cytoplasmic and tissue components by virtue of these components having properties (such as viscoelasticity, surface tension and density inhomogeneities) in common with ordinary non-living materials. If excitable media (Ross *et al.* 1988) are included among such materials, chemical waves can be added to the list of patterning effects exhibited by both nonliving and living systems.

Individual gene products, e.g. RNA and protein molecules, are of course subject to generic *chemical* effects, such as nucleophilic displacement (Cech, 1985) and hydrophobic interactions (Chothia, 1984) in achieving their functional morphologies. We suggest that increased size, chemical heterogeneity, and content of macromolecules biosynthetically 'distant' from primary gene products (e.g. glycosaminoglycans), have made living systems increasingly subject to generic *physical* effects. These in turn may have opened up new pathways for evolution (Comper, 1990), laying the basis for tissue morphogenesis and pattern formation.

It is not only that generic physical processes *might* be used during morphogenesis and pattern formation: in many cases it would take special mechanisms to *prevent* these effects from influencing biological outcomes. Organisms may indeed incorporate examples of such mechanisms: one function of the cytoskeleton may be the prevention of nuclear sedimentation (Moroz, 1984). However, it is reasonable to expect that generic physicochemical effects would often be used, rather than opposed, by living systems. It is also significant that virtually all morphogenetic and patterning effects seen in developing systems (e.g. epiboly, invagination, involution, ingression, delamination, microfingering, striping; see Gilbert, 1988) can, in principle, have originally arisen by such generic effects.

The generic processes discussed above are capable of interacting with one another in subtle and unexpected ways. Convection in a multicomponent system can arise from variations in interfacial tension, density or viscosity, individually or in concert, and in many cases unravelling the contributions of these effects is technically difficult (Napolitano, 1984). As discussed above, chemical potential differences can drive density inversions, leading to gravity-driven convection in systems ostensibly in gravitational equilibrium (Comper *et al.* 1987*b*). Moreover, reaction–diffusion systems with intrinsic pattern-forming capability are exquisitely sensitive to the presence of gravitational fields, which can influence the pattern of chemical waves attained at steady-state (Kondepudi and Prigogine, 1983). Because of the chemical complexity of eggs, and of developing systems in general, it would be expected that multiple generic effects could contribute (along with locally acting genetically specified molecular interactions) to bringing about specific morphological outcomes.

Our overall viewpoint has some affinities to that developed by D'Arcy Thompson in his book *On Growth and Form* (1942, 1961). This author was also concerned with the imprint of physical forces on biological form and pattern, and with distinctions (which he acknowledged, were not always easy to make) between 'properties of the organism...that are physical in origin and those that are *sui generis* and peculiar to living things' (D'Arcy Thompson, 1961; p. 13), a dichotomy that roughly corresponds to our distinction between generic and genetic processes. But D'Arcy Thompson wrote virtually nothing about mechanisms of development; his most compelling examples, such as similarities in the arrangement of bone trabeculae to

the weight-bearing girders of a cantilever bridge, or the possibility of deriving body or skull structure of one species from another by coordinate transformation, are rationalized on the basis of forces acting on adult forms. They provide little insight into the mechanistic bases of the ontogeny of these properties. The most important deficiency in D'Arcy Thompson's analysis, however, is his lack of serious consideration of mechanisms of heredity, and in particular, of how generic physical forces could act in a mutually reinforcing fashion with genetic processes in giving form to organisms.

More recent discussions of the role of physical and physicochemical processes in development (e.g. Meinhardt, 1982; Harrison, 1981), like that of D'Arcy Thompson, have taken for granted the ability of hereditary mechanisms both to provide the necessary ingredients for, and to incorporate the effects of, these processes. Mittenthal (1989) has gone beyond this by explicitly considering a 'principle of matching' in which genetically specified outcomes that meet certain physical constraints are most likely to be preserved by evolution (this is similar to the 'Baldwin effect' (Simpson, 1953)). By assuming that generic effects are efficacious developmental determinants at at least some points in an organism's evolutionary history, our perspective provides a reasonable way for this matching to come about.

Other general frameworks for morphogenesis and pattern formation have deemphasized generic effects while placing more of a burden on the efficacy of presumed genetic programs. 'Positional information' (Wolpert, 1969) is a model for pattern formation that hypothesizes a genetically based potential of each cell in an embryonic field to assume a biochemically unique, site-specific state on the basis of simple cues provided by monotonic gradients of signal molecules. The 'morphoregulator hypothesis' (Edelman, 1988) invokes a hierarchy of rigidly programmed events in which the establishment of tissue boundaries and organ form depend exclusively on highly specific molecular interactions.

In contrast to genetic programming hypotheses, which can potentially account for any imaginable form or pattern, we have proposed an analysis of development that embodies generic mechanisms that act on tissues and their components, either during the modern ontogeny of an organism, or on its ontogeny sometime in its evolutionary history. Changing patterns of gene expression during development can drive morphogenesis and pattern formation by making tissues responsive to fresh generic effects. Genetic change during evolution can act to conserve and reinforce these morphogenetic tendencies, or in rare instances, set phylogeny on a new path by establishing susceptibility of the embryo or its tissues to different generic forces. Such generic–genetic interactions will not give rise to all conceivable forms and patterns that may be constructed from living cells and biological macromolecules. They may nonetheless provide a concrete account of why organisms achieve the particular variety of forms with which we are so familiar.

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