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Generic physical mechanisms of tissue morphogenesis: A common basis for development and evolution

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Abstract

Morphological evolution is usually considered to occur by the selection of small heritable variations in the expression of anatomical traits, on the basis of improved adaptation to new environmental conditions. An alternative mode of morphological evolution is proposed here: the production of a spectrum of forms by the action of intrinsic physical properties of cell aggregates, followed by intense selection for biochemical mechanisms that make the generation of a subset of viable morphologies, and pathways of transition between morphologies, more reliable. This view provides an account of the origins of important features of metazoan body plans and organ forms, including gastrulation and other types of tissue multilayering, lumen formation, and segmentation. It also implies that most major morphological innovations would occur early in phylogeny, often more than once, with much subsequent genetic selection being of a stabilizing or canalizing variety. Consistent with recent findings, this view predicts that functional redundancy among developmentally important genes and genetic circuits should be prevalent.

Introduction

Morphological change in organisms is generally held to occur in two entirely distinct ways. Form is said to change in a "programmed" fashion during development, as a result of a well-ordered progression of gene activities. In contrast, changes in form that occur during evolution are considered to be randomly generated, with no preferred pathways intrinsic to the evolving organism itself. Although living tissue are, in general, semi-solid, deformable materials – "soft matter" (de Gennes, 1992) – it is interesting that biological accounts of the

production of form make little or no reference to the relatively well-understood physical processes which mold such materials. Whereas the effects of gravity, surface tension, and air resistance on the behavior of liquids are sufficient to account for the fact that any two raindrops have shapes that differ in exactly the same way from that of a sessile droplet of mercury, the explanation for why any two *Xenopus* embryos have morphologies that differ in the same way from a *Drosophila* embryo is considered to be another story entirely.

Because organisms typically resist, rather than conform to, changes in their physical environments, physical accounts of morphological variation have rarely been invoked by developmental or evolutionary biologists. D'Arcy Thompson (1942) was an exception. He noted that body forms of different fish species, for example, could be "deformed" into one another by simple coordinate transformations that plausibly derived from environmentally-related differentials in mechanical stress. But D'Arcy Thompson failed to explain how such deformations could be inherited, and his ideas had little impact on the mainstream of biological thought.

In this article I consider the possibility that physical determination of biological form, of the sort considered by D'Arcy Thompson, was important in establishing body plans and organ morphologies during *early* stages of metazoan evolution, *i.e.*, before the acquisition of redundant developmental pathways over the course of evolution guaranteed that variations in the physical environment had only minimal effects on morphological outcome. All processes that lead to changes in tissue form are, of course, ultimately "physical". But among a tissue's physical properties we can identify those characteristics such as cohesivity, viscosity, and elasticity that would always have been present, regardless of the details of the tissue's biochemical makeup, and are therefore "generic" to such materials (Newman and Comper, 1990; Forgacs et al., 1991; Newman, 1992; Forgacs and Newman, 1994). Generic physical properties define a tissue's susceptibility to a group of common physical effects. Because these properties are transmitted by multicellular organisms to their descendents, any aspect of morphology that is determined by them is heritable, but only roughly so. This is because physical effects are subject to variations in the physical environment, *e.g.*, temperature, pH, salinity.

It is reasonable to suppose that the earliest metazoa lacked much of the molecular machinery that "overdetermines" the choice of developmental pathways in modern organisms. In the absence of these stabilizing mechanisms, generic physically-based morphogenetic mechanisms, subject to variable environments, would have been especially protean in their effects, giving rise to an array of biological forms and patterns that could virtually have exhausted the morphological possibilities of tissue-based systems. Subsequently, as a result of both function-based natural selection and intrinsic propensities of gene-based regulatory mechanisms to become linked in particular ways, a subset of "physically-templated" morphologies could have been stabilized, reinforced, and refined, by the evolution of elaborate fail-safe mechanisms. The development of each biological "type" would thereby have become less subject to the physical molding forces that first defined its form. Moreover, genetic mutations in redundantly determined organisms

would have had decreasing morphogenetic effect, and phylogenetic stasis would have become the norm.

If these suggestions are valid, they could help establish causal links between ontogeny and phylogeny, and provide an account of the origin of biological form often noted to be missing from classical statements of selectionist theory (Butler, 1878; Galton, 1894; Ho and Saunders, 1979; Goodwin, 1982; Oyama, 1985). This view can also help interpret the growing number of studies that point to functional redundancy in physiological and developmental regulatory processes (Brookfield, 1992; Tautz, 1992), and provide insight into the relationship of this redundancy to the tempo and mode of evolutionary change (Eldredge and Gould, 1972).

Stereotypical consequences of differential adhesion

Hundreds of gene products and other molecules have been identified that influence, affect, or "play a role" in changes of tissue form and pattern during embryogenesis. What is striking, however, is how limited in kind are the outcomes of these developmental processes, despite their molecular complexity. Multicellular organisms always start out as clusters of cells. Individual cells may detach from one place and reattach elsewhere, or entire tissue layers may separate from one another. A tissue layer can engulf or be engulfed by its neighboring layer, undergo segmentation, form a hollow ball, or roll up into a tube. These morphological outcomes can, in principle, all be generated by *cell adhesivity* and its modulation, the precise identity of the adhesion molecules being less important than their capacity to cause cells to stick together. For example, regardless of whether cells adhere to one another using a calcium-independent homophilic integral membrane adhesion protein such as N-CAM (Edelman and Crossin, 1991; Edelman et al., 1987), or a calcium-dependent one such as L-cadherin (Takeichi, 1991), by means of a heterophilic integral membrane protein system such as $\alpha_1\beta_2$ integrin/ICAM (Hemler, 1990; Rudnicka, 1992), or an integral membrane protein-extracellular matrix system such as $\alpha_5\beta_1$ integrin/fibronectin (Akiyama et al., 1990), the morphological result is always a cluster of cells.

Where more than one adhesion system comes into play, or where a single adhesion system is modulated in strength, cells can detach from the main aggregate, or "compartments" can form. This is a phenomenon by which distinct spatial domains are established within a single tissue, with no interchange or mixing of cells across the common boundary (Garcia-Bellido, 1975; Crick and Lawrence, 1975). Compartmentalization in tissues has been considered to arise from essentially the same physical principles as *phase separation* (*e.g.*, oil *vs* water) in liquids (Steinberg, 1978; Steinberg and Poole, 1982; Armstrong, 1989).

Detachment of cells and the formation of compartments provide opportunities for different regions of a multicellular aggregate to follow distinct fates, with respect to both localization and specialization. The capacity of tissues to move actively, or to flow passively, in response to adhesive differentials at their compartmental

interfaces, can generate morphological variation, such as engulfment of one compartment by another.

In all multicellular organisms with more than a single tissue layer, *gastrulation* is the stage in development in which the distinct layers are established. Gastrulation falls into five main types, with some embryos making use of more than one type. All five modes of gastrulation could, in principle, be achieved simply by modulation of adhesivity in different regions of the embryo. In *invagination*, a ball of cells moves into the cavity of a hollow blastula. *Epiboly* and *involution* both involve the envelopment of a distinct vegetal or marginal population of cells by the spreading ectodermal layer. *Delamination* occurs in solid blastulae when an outer layer of cells separates from the inner cell mass, and *ingression* involves the independent detachment of cells from the blastula wall and their accumulation within the cavity. (See Gilbert, 1991 and Willmer, 1990 for reviews).

Segmentation is the demarcation of tissue primordia into a linear arrangement of structually similar domains. This is seen, for example in the establishment of body segments in insects (Turner and Mahowald, 1977; Karr et al., 1989), of the blocks of bone- and muscle-forming mesoderm, termed somites, along the embryonic axis of vertebrates (Hamilton, 1969; Stern and Keynes, 1987), and of the periodic swellings in the vertebrate hindbrain, termed rhombomeres (Guthrie and Lumsden, 1991). Like the various forms of gastrulation, the different types of segmentation can be viewed as manifestations of differential adhesion. For if an aggregate of cells were capable of generating a series of compartments with alternating adhesive properties, a segmental body plan would result (Newman, 1993a).

We can also consider the consequences of adhesive differentials on the surfaces of *individual* cells. In particular, if a region of each cell in an aggregate became nonadhesive to its neighbors, perhaps by the targeting of an anti-adhesive protein to a portion of the cell surface, lumens would inevitably form, leading to saccular or tubular structures (Tsarfaty et al., 1992).

Taken together, the processes of detachment, compartmentalization, multilayering, segmentation, and lumen formation can generate a wide variety of body plans. Nevertheless, the morphological outcomes, even those that can be conjured up by the application of an arbitrary sequence of the processes mentioned, would all be unmistakably organisms. None could be confused with a calculator or an internal combustion engine. Put another way, regardless of which adhesive molecules have been used in phylogeny, and by what various means adhesivity has been modulated, major features of all metazoan body plans, past and present, can, in principle, have been generated by the spatiotemporal regulation of cell adhesion.

Generic physical mechanisms of pattern formation

The fact that a wide variety of organismal forms, including some that never existed, or no longer exist, can be produced by a "simple" physical property of tissues, namely intercellular adhesion and its modulation, alerts us to the vast morphogenetic potential latent in even the simplest multicellular aggregate. *Cohe-*

sivity, the physical consequence of intercellular adhesion, is clearly a generic property of tissues and as such, is transmitted from any multicellular aggregate to any other one derived from it. Spatial variations in cohesiveness within an aggregate can lead to compartment formation, tissue multilayering such as gastrulation, lumen formation, or segmentation. However, if such spatial variations were simply a random occurrence, any form so generated, whether or not it was well-adapted to its surroundings, would be transient, since it would be incapable of passing on the conditions of its formation to its progeny. It is significant, therefore, that there are certain additional generic physicochemical properties of multicellular aggregates that potentially enable them to propagate definite spatial patterns of intercellular adhesion to their "offspring".

Sedimentation of cellular materials is one simple way that this can be accomplished (Radice et al., 1981). Of the variety of molecular components produced or taken up by cells, some are denser than the average density of cytoplasm. If the founder cell of a multicellular aggregate contained a sedimenting component, such as yolk platelets, then this product would, by virtue of gravity, become asymmetrically distributed in the cluster that resulted from division of the original cell (Fig. 1B). If, in turn, each cell's capacity to produce its characteristic adhesion molecules were influenced by the presence of the sedimenting component, the aggregate would then exhibit regional adhesive differences. The result would be compartmentalization, which would typically lead to a gastrula-like outcome (Fig. 1F). This morphological consequence of the linking together of the processes of differential adhesion and intracellular sedimentation would "breed true" from generation to generation, since the biologically reproduced materials would, for physical reasons, always behave in essentially the same fashion.

The reacting and diffusing chemicals within cells and tissues provide the conditions for several other generic processes that can give rise to molecular nonuniformities in space and time. *Gradients* of chemical composition can be formed by diffusion from a localized source (Fig. 1D), which may be established when an initially isotropic founder cell interacts with a chemically or physically nonuniform environment. The diffusing molecule could be passed directly from cell to cell by transport across membranes (Crick, 1970), or indirectly, through the extracellular space (Newman and Frisch, 1979). If a nonuniformly distributed chemical happened to influence the expression of adhesivity (Fig. 1A) in the cluster of cells produced by this founder, reproducible compartmentalization and morphogenetic change would result (Fig. 1F,H).

Cellular metabolism would have included many coupled chemical reactions even in the pre-metazoan stages of evolution. Such reactions, when present in multicellular aggregates, are capable of becoming organized in time and space in ways that could lead to morphological changes. For instance, positive feedback of the production of a chemical species, in the context of an otherwise self-limiting set of reactions, will often give rise to temporal oscillations in the concentration of one or more of the reactants (Fig. 1C) (Goldbeter and Lefever, 1972; Norel and Agur, 1991; Tyson, 1991). Such chemical oscillations which are well-known experimentally (Chance et al., 1964; Nurse, 1990; Hall and Rosbash, 1993), are generic properties

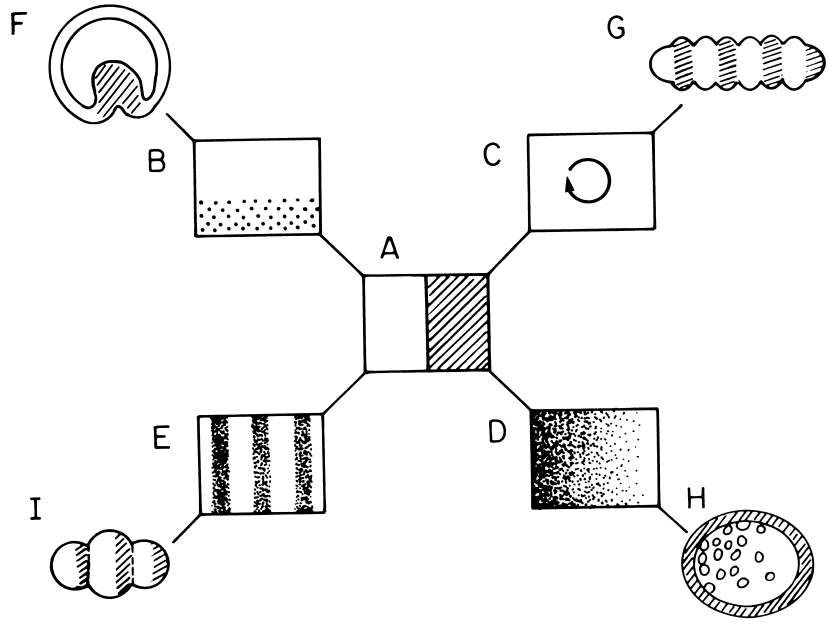

Fig. 1. Schematic representation of consequences of linking the regulation of cell-cell adhesion to various physical and chemical pattern-forming mechanisms. The effect of differential adhesion (A) is to cause a boundary to form within the tissue mass, across which cells will not mix. Sedimentation of a dense cytoplasmic component (B) is a way in which an adhesion-regulating molecule can become non-uniformly distributed in a founder cell. Other mechanisms generating spatial non-uniformities include gradient formation (D) and reaction-diffusion coupling (E). Temporal non-uniformity in a regulatory molecule can be achieved by an oscillatory kinetic scheme (C). Mechanisms B and D can both generate adhesive differentials in a localized region of tissue, leading to engulfment of the more cohesive by the less cohesive region (F), or dispersion of a less adherent population of cells (H). High (or as shown, low) concentrations of a signal molecule can also trigger the targeting of an anti-adhesive molecule to a portion of the cell surface, causing lumen formation (F,H). Outcomes F and H represent two of several types of gastrulae. Mechanisms C and E can both lead to periodic alternations in adhesive characteristics, causing segments to form (G,I).

of networks of chemical reactions: they can occur in countless different reaction systems, which need only fulfill a set of formal kinetic conditions in order for temporally periodic changes in concentration to occur. If a molecule which functioned as a regulator of cell-cell adhesion were to undergo temporal oscillations in concentration, it could give rise to a pattern of bands of tissue with alternating adhesive characteristics (Fig. 2A). Segmentation can thus arise from the combined effects of two generic physical processes: chemical oscillation and differential adhesion (Newman, 1993a); (Fig. 1G).

The joint effects of positive autoregulation and cross-inhibition in a chemical reaction system with diffusion, within a confined domain, such as a tissue mass, can

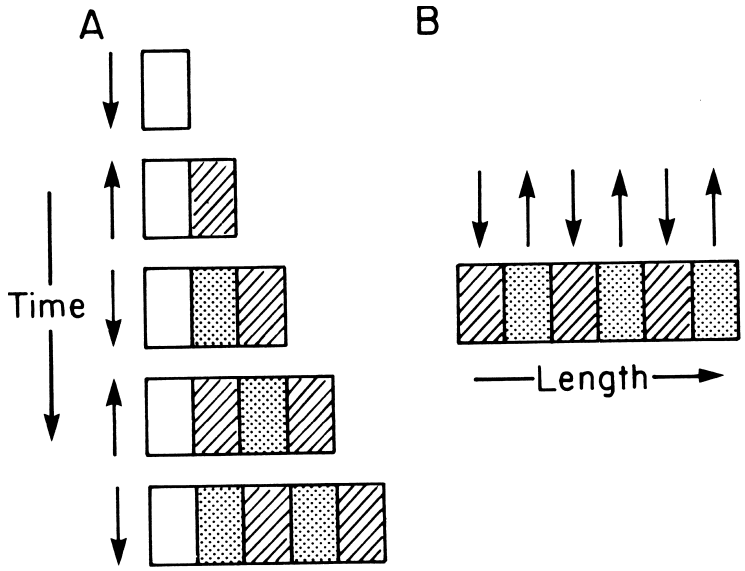

Fig. 2. Mechanisms of segmentation involving regulation of cell-cell adhesivity by a temporally or spatially periodic signal molecule. In (A), a temporal oscillation of an adhesivity regulatory molecule occurs in a localized growth zone (empty box). When the regultory molecule is present in low concentrations (down arrow), division in the growth zone yields cells with one of two possible adhesive characteristics (hatched boxes). When the regulatory molecule is present in high concentrations (up arrow), cells of the alternative adhesive characteristic are produced (stippled boxes). In (B) the high and low concentrations of the regulatory molecule are present simultaneously across the length of the tissue, leading to bands of tissue with alternating adhesive characteristics. (See Newman (1993a) for additional details.)

lead to an unusual form of spatial organization: stable, nonuniform patterns of concentration of one or more of the molecules. This phenomenon, which was first hypothesized by Turing (1952), and has now been demonstrated experimentally in several chemical systems reacting within semi-solid media (Castets et al., 1990; Ouyang and Swinney, 1991; Lengyel et al., 1993), can lead to stripes, spots or even spirals (Epstein, 1991) of molecular concentration (Figs. 1E, 3A). Like the temporally periodic signals discussed above, these spatially periodic signals, if linked to the regulation of intercellular adhesion, can also lead to segmental (Fig. 1I; 2B) or other periodic forms of tissue organization (Newman and Frisch, 1979; Newman et al., 1988; Newman, 1993a, b).

The strategy of the genes

Two interdigitated sets of evenly spaced chemical stripes indeed arise during the early blastoderm stage of *Drosophila* development, when thousands of nuclei reside

in a common cytoplasm. These correspond to the distributions of the "pair-rule" proteins, even-skipped (Frasch et al., 1987) and fushi tarazu (Weir and Kornberg, 1985). By regulating cell-cell interactions after cellularization of the embryo, the pair-rule proteins map out a pattern of fourteen segments in the fly larva (Lawrence et al., 1987; Ingham et al., 1988). Is the "chemical prepattern" for these segments established by one of the generic processes discussed above? The potential for this is at least implicit in the biochemical circuitry of the *Drosophila* embryo. Both even-skipped and fushi tarazu are transcription factors which can diffuse between nuclei in the syncytial blastoderm (Frasch, 1987; Lawrence et al., 1987). Moreover, both of these proteins are positively autoregulatory (Harding et al., 1989; Ish-Horowicz et al., 1989; Schier and Gehring, 1992). The segmental prepattern of *Drosophila* therefore not only resembles the outcome of a generic reaction-diffusion mechanism, but some of its key components are regulated in a fashion consistent with their participating in such a mechanism. However, it has become increasingly clear that the deceptively simple even-skipped and fushi tarazu stripes are in fact established by a highly complex set of interactions involving numerous other transcriptional factors, pre-existing gradients and multiple gene promoters (Stanojevic et al., 1989; Goto et al., 1989; Small, 1991; Frasch and Levine, 1987).

Since it is highly improbable that this elaborate genetic machinery for producing segments evolved all at once in a non-segmented ancestral form, we are left with two alternatives: the even-skipped and fushi tarazu stripes could have evolved one by one, by random mutation of the relevant genes, stopping at precisely interdigitated sets of seven bands of each factor because of the decisive adaptational advantage of fourteen segments in the adult fly. Or, as suggested by the discussion above, one or both patterns of seven stripes could have arisen virtually at one stroke, given the materials at hand, and the relevant physicochemical patterning processes. This second possibility has the benefit of producing a criterion for subsequent genetic evolution, namely, preservation of a viable morphology in which the "end point" is implicit from the beginning. It also permits us to dispense with far-fetched adaptationist scenarios for why organisms are constructed as they are (Gould and Lewontin, 1979).

The diverse organismal forms that may be produced by the combination of various generic physical mechanisms acting on multicellular aggregates differ in at least one important way from the outcomes of processes of biological development in modern organisms. Because physical mechanisms are influenced by physical parameters, an embryo which was entirely dependent on gravity, diffusion, chemical oscillations, or reaction-diffusion coupling, to establish its body plan, would develop differently if its orientation were altered, or its ambient temperature or the chemical composition of its environment were changed. A recently studied chemical reaction-diffusion system, for example, underwent a transition from a spatially uniform state to a hexagonal pattern of spots when the temperature was decreased below 18° C, and developed a stable pattern of stripes, rather than spots, when the input concentrations of two of the reagents were slightly increased (Ouyang and Swinney, 1991). In contrast, pathways of biological development, however much their outcomes may resemble those of generic processes, are "canalized": the

developmental process can often get "back on track" even after severe perturbations (Schmalhausen, 1949; Waddington, 1957).

The idea that development proceeds with numerous back-up mechanisms that make it resistant to perturbations – what Spemann referred to as "double assurance" (Hamburger, 1988) – is familiar to developmental biologists. The question of which processes are being backed up, and which are doing the backing up, would seem to be a central one for evolutionary theory, but is difficult to address within the conventional neo-Darwinian framework. In particular, if the mode of morphological evolution is considered to be fortuitous, incremental and open-ended, there is no *a priori* way of identifying preferred forms, *i.e.*, those variants that will crystallize out of all the morphological noise by recruitment of stabilizing biochemical circuitry. The view proposed here suggests that the preferred forms are none other than the multilayered, segmented, vesiculated entities produced by the concatenation of generic tissue processes during the early evolution of the metazoa. Of this spectrum of possible morphologies, a subset of viable ones would have persisted within changing environments if their production became linked to genetically-specified reinforcement processes in addition to the generic physical mechanisms that originally brought them about.

To see how this could happen, consider a set of patterns of a particular tissue regulatory protein which may be generated by a reaction-diffusion mechanism (Fig. 3A). For simplicity we will assume the regulatory protein to be a transcription factor acting within a syncytium, and that the positive autoregulation required for pattern formation is exerted at the transcriptional level, by the factor's binding to its own promoter. This is analogous to the situation found for even-skipped and fushi tarazu (Harding et al., 1989; Ish-Horowicz et al., 1989; Schier and Gehring, 1992). In principle, there is no reason why any of the patterns shown in Fig. 3A could not specify a viable arrangement of tissue elements, or body plan, but clearly those forms which were reliably passed on to subsequent generations would tend to increase in number. As noted above, transitions between these different patterns can be induced by variations in environmental parameters, so that forms which were generated in this purely physical way would be inherited in an unreliable fashion.

Now let us assume that promoter duplication occurs, and that one or more of the redundant promoters mutates to a condition of being activated by a particular concentration of a different, nonuniformly distributed, molecule that originally had nothing at all to do with the generation of the pattern in question (Fig. 3B). Any such arrangement that led to "ectopic" production of the transcription factor (*e.g.*, between the original stripes) would disrupt the dynamical process and lead to erratic morphological outcomes. In contrast, if a concentration-sensitive promoter were to reinforce one of the physically-templated patterns, as a whole, or in part (Fig. 3C), it would make that pattern more heritable. The subpopulation of organisms containing this back-up mechanism would become more prevalent, although there would be no morphological change associated with it.

In addition to indicating how additional genetic mechanisms can come to reinforce those containing a "generic" component, this example also demonstrates

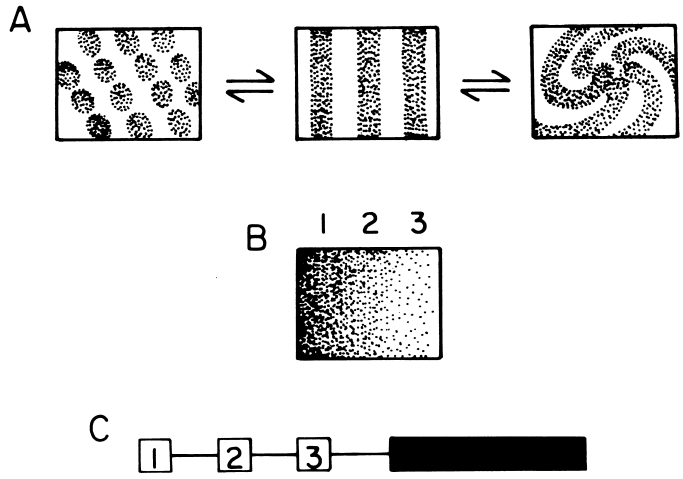

Fig. 3. Model for selective reinforcement of a reaction-diffusion pattern by promoter duplication. Positive autoregulation of the gene (solid bar in (C)) specifying a transcription factor which diffuses in a syncytium leads to several discrete but interconvertible distribution patterns of the factor (A). One or more rounds of promoter duplication gives rise to a set of promoters (C; 1–3) which are activated by differing concentrations of a graded molecule originally having no role in the generation of the pattern (B; 1–3). If the graded molecule now regulates the gene in a way that corresponds to the original concentration pattern of the first factor, reinforcement of the pattern will occur. Of the patterns in (A), only the striped one can be readily reinforced by this mechanism.

the principle that some generically-templated patterns are inherently more susceptible to stabilization by genetic regulatory mechanisms than others. In contrast to the pattern of stripes in Fig. 3A, which is periodic in only one dimension, and thus can be reinforced by a single variable, such as a monotonic gradient, the spot and spiral patterns would require at least two orthogonal gradients, and a formidably complex set of concentration-sensitive promoters, to reinforce them by gene regulatory circuits. Among the three patterns, therefore, the one composed of repeated stripes would have been most likely to persist evolutionarily, not necessarily because of any functional advantage, but because it is more readily reinforced by randomly acquired genetic circuitry.

In the *Drosophila* embryo, the formation of certain even-skipped and fushi tarazu stripes is dependent on the presence of graded "maternal effect" gene products, such as bicoid, and locally concentrated "gap" gene products, such as hunchback (Frasch and Levine, 1987). The scenario described above, in which a pattern of protein distribution first determined by a reaction-diffusion process comes to depend on the distribution of proteins incidental to the original mechanism, can serve as a model for what may have taken place during the evolution of the segmentation system in *Drosophila*.

Physical templating of organogenesis

Once the major features of metazoan body organization became established and genetically reinforced, the locus of further morphological evolution would have shifted to small tissue primordia which were susceptible to many of the same generic physical effects that are proposed to have guided the organization of body plans. The organs of metazoan organisms, like embryos at their earliest stages, start out as clusters of cells. They turn into glands, hearts, limbs, etc., in large part by the processes of compartmentalization, multilayering, segmentation, and lumen formation that give rise to the basic body plans. Like the inheritance of the body plan as a whole, the inheritance of the capacity to reproducibly form organs would appear to require not an abstract genomic representation of these structures, but rather the production of tissue masses subject to appropriate generic physical effects.

Specialization of cell types for functions other than adhesivity (*e.g.*, support, contractility, excitability) is a feature which evidently took on increasing importance as organs became more elaborate. This capacity to *differentiate* may have had its origin at the inception of the metazoa, in transcriptional control mechanisms that ensured reliable differential expression of adhesion molecules, as suggested above. But in addition to a role in establishing the morphological characteristics of organs by modulating adhesion, it is likely that such mechanisms could have been mobilized for other purposes once organ primordia relatively independent of the rest of the body had emerged. These roles can have included differential expression of genes specifying proteins with functions other than adhesion, such as myosin, actin, ion transporters, etc., either quantitatively, or (after gene duplication and divergence), qualitatively. Thus organs would come to be characterized not only by their morphological characteristics, but by their functional differentiation.

The coordinate action upon small tissue primordia of generic developmental processes identical to those proposed to have templated the body plans, and the synthesis of cell-characteristic products, may thus account for the origin and particular forms assumed by accessory organs. An example is the vertebrate eye, in which a lumenal structure formed by an extension of the forebrain (the optic cup) partially envelops a vesicle formed by the head ectoderm (the lens primordium). Consequent inductive interactions passing between the neural and ectodermal tissues signal the latter to produce the crystallin proteins characteristic of the lens (Grainger, 1992). Another example is the vertebrate limb, in which segmentation of the limb bud mesoderm into arrays of skeletal primordia along both the proximodistal and anteroposterior axes is accompanied by the elaboration of cartilage-specific extracellular matrix components in the mesodermal segments (Newman, 1988; 1993b).

Evolutionary consequences of genetic-physical interaction

The previous discussion suggests that the embryonic organization of organisms is largely a predictable function of the materials of which they are composed and the

physical world of which they are a part. Mechanisms of morphogenesis and pattern formation are tied to particular sets of molecules insofar as those molecules participate in certain generic *physical* functions: adhesion, sedimentation, diffusion, positive autoregulation. At the early stages of multicellular evolution genetic change could shift the balance among these various physical processes, and promote the elaboration of possible metazoan morphologies. But after this initial phase, it is proposed that the most important role of genetic change in morphological evolution would be the stabilization (Schmalhausen, 1949), canalization (Waddington, 1957), or reinforcement of physically-templated body plans and organ forms. This suggestion sheds light on a number of otherwise puzzling aspects of the evolution of form.

Homoplasy. The morphological similarity of an anatomical feature in divergent lineages whose common ancestor was not similar to either of the lineages in this trait, is referred to as *homoplasy* (Wake, 1991). There are many notable examples of this phenomenon: Arthropods and vertebrates are both segmented, but their common ancestor almost certainly was not (Willmer, 1990; Bateson, 1894; Holland, 1990). The eyes of molluscs and vertebrates are constructed in very similar ways, but arose independently well after the evolutionary divergence of the two taxa. In three independent lineages of plethodontid salamanders there has been a reduction in the number of hind limb digits from five to four (Wake and Larson, 1987). The prevalence of homoplasy in invertebrate phylogeny is particularly striking (Willmer, 1990). Such occurrences have traditionally (within the neo-Darwinian framework) been considered to result from convergent evolution, based on functional adaptation to similar environments. Such explanations sometimes stretch credulity, and alternative interpretations, that some morphological features may reflect "developmental constraints" (Gould and Lewontin, 1979; Maynard Smith et al., 1985), "side effects" (Müller, 1990), or "recurrent production of discrete alternative phenotypes that are intrinsic to the generative system" (Wake and Larson, 1987), seem more reasonable.

The concept of templating of biological form by generic physical mechanisms goes even further, by proposing that the generative systems of development are themselves intrinsic to the material properties of early embryos and organ primordia. Homoplasy, in this view, is the result of exploration by tissue masses (organisms and their organs) of a delimited universe of possible morphologies. If this is the case, recurrence of anatomical forms across taxa is virtually inescapable, and unlike scenarios involving convergent evolution, not dependent on improbable conjunctions of historical accidents.

Discordance between genetic and morphological evolution. Homoplasy is only one manifestation of this phenomenon, which is a major problem for the neo-Darwinian synthesis. A recent laboratory study, for example, analyzed inherited differences in the morphology of the mandible in inbred strains of mice, in relation to the genetic divergence between the strains. It was concluded that there was little correspondence between morphological and genetic divergence (Atchley et al., 1988).

The Great Lakes of East Africa are microcosms of such discordances. For instance, the cichlid fish of Lake Victoria radiated into several hundred morpholog-

ically distinguishable species over a period of 200 000 years with only minor genetic change (Meyer et al., 1990). Conversely, a lineage of several species in Lake Tanganyika exhibits six times more genetic variation than the entire Lake Victoria species flock, but virtually no morphological divergence (Sturmbauer and Meyer, 1992).

If morphological evolution is considered to be driven largely by generic properties of tissues, with random genetic change acting to incline the developing system along one or another of the generically-templated pathways, these discordances present no problem. It is clear that the relationship between genotypes and morphological phenotypes prescribed by this view is many-to-many. For example, variations in habitat (*e.g.* temperature, pH) that have consistent effects on certain reaction rates, or cell-cell adhesive strengths, important in morphogenesis or pattern formation, can potentially lead to reproducible anatomical differences in the affected subpopulations. Minor genetic variations can act to stabilize one or another of the developmental pathways. The resulting morphologically and ecologically distinct, but genetically-similar, subpopulations would satisfy the criteria for being separate species. Any substantial genetic divergence between these lineages would follow, rather than precede, speciation. Alternatively, certain organismal forms may be particularly suited to the recruitment of independently-evolved biochemical stabilizing mechanisms (see, for instance, the example in Fig. 3). This may have the effect of giving rise to genetically diverse (and therefore reproductively isolated) descendants with virtually identical appearance and behavior.

'Explosive' morphological evolution and punctuated equilibria. The Neo-Darwinian view of morphological evolution implies that forms of increasing complexity should have appeared gradually over time, at a pace corresponding to typical rates of genetic change. This expectation conflicts with the fossil record, insofar as morphological disparity (*i.e.* divergence of body plans) appears to have reached a maximal level early in this history of metazoan life. For example, the Burgess Shale of British Columbia, deposited in the early Cambrian period, contains fossils of structurally elaborate hard-bodied organisms representing virtually all of the body plans present today, about 530 million years later, and possibly additional ones (Whittington, 1985; Conway Morris, 1989; Gould, 1989; Briggs et al., 1992; Foote and Gould, 1992; Briggs, 1992). All the morphological disparity contained within the Burgess Shale, moreover, was probably generated during the 100 million years or so that separated the "Cambrian explosion" from the earlier Vendian or "Ediacaran" metazoa: flat, often hollow and/or segmented organisms that are difficult to assign to any extant taxonomic groups (Glaessner, 1984; Fedonkin, 1985; Conway Morris, 1993).

The view of morphological evolution presented above actually predicts that an initial profusion of body plans would have characterized the early stages of metazoan life. Before certain designs were "locked in" by the acquisition of stabilizing molecular mechanisms, morphogenetic and pattern forming processes would have been more variable in their effects, giving rise to lineages each of which inherited an array of morphologies rather than a uniquely determined body plan.

The phenomenon of *stasis* – the perseverance of some organismal forms over vast periods of time despite major environmental and ecological changes – is even more of a problem for standard neo-Darwinism than are the bursts of morphological innovation that evidently produced the basic body plans, and certain organs such as the vertebrate limb (Hinchliffe and Johnson, 1980; Newman, 1984). The reason is that the relatively sudden emergence of viable novelties (the "hopeful monster", in Goldschmidt's terminology (Goldschmidt, 1940)), while relegated to a minor role, is not excluded by Darwinian theory. In contrast, the continual emergence of small morphological variations, which are supposed to provide raw material for incessant incremental change by means of natural selection, is an absolute requirement of the theory. In accounting for stasis, Darwinians have sometimes appealed to a hypothetical "cohesion of the genotype" (Mayr, 1988), the assumption being that by the time the complex genetic machinery needed to produce a given structure has evolved, the system is so highly integrated that even a small degree of evolutionary tinkering would be fatal. However, there is no evidence for such unique integration in the genetic systems that participate in morphogenesis, and recent experiments in which apparently key regulators of morphogenesis are "knocked out" with little or no effect (see below) actually argues against the "cohesion model".

The view described here, suggesting a rapid elaboration of morphogenetic possibilities during the early stages of evolution of body plans and organ forms, followed by the selective loss of some forms, and the locking in and minor modification of others by genetic reinforcing mechanisms, implies that evolution will proceed by fits and starts of morphological innovation, followed by stasis of surviving forms. The proposal that "punctuated equilibrium" represents the actual tempo and mode of evolution, rather than an artifact of an incomplete fossil record (Eldredge and Gould, 1972), is consistent with a number of paleontological studies (*i.e.*, Williamson, 1981; Cheetham, 1986; Stanley and Yang, 1987). The interplay between generic developmental mechanisms and the natural selection of genetic reinforcing processes provides a mechanistic interpretation of such patterns of evolutionary change, and indicates the types of organismal features for which it might be expected to hold.

Genetic redundancies and developmental canalization

If, as suggested, phylogenetic stasis results from the accretion of mechanisms that stabilize and reinforce particular morphological outcomes, modern organisms should contain extensive functional redundancies in the regulatory networks that specify developmental pathways. Targeted mutagenesis experiments have indeed provided evidence that there are families of closely related genes in which individual members are functionally interchangeable during development (*e.g.*, the *Enhancer of split* locus in *Drosophila* (Campos-Ortega and Knust, 1990)), and that other developmentally important gene products (*e.g.* MyoD (Rudnicki et al., 1992) and tenascin (Saga et al., 1992) in mice) can be functionally replaced by more distantly

related, or even unrelated, molecules. In still other cases, like that represented by the interaction of the *nanos* and maternal *hunchback* gene products in *Drosophila* (Hulskamp et al., 1989; Irish et al., 1989), molecules apparently can act in concert to help bring about a developmental outcome (*e.g.*, segmentation) that will occur even if the two products are absent. Functionally redundant molecules and circuitry are characteristic not only of developmental processes, but of other aspects of cell physiology as well. For example, progression to the S phase in the yeast *S. cerevisiae* is controlled by the three G1 cyclin genes together, or by any one of them alone (Reed, 1991).

True redundancy is difficult to accommodate within the neo-Darwinian framework since a mutation that compromised the function of one of the genes would not be selected against, and drift would eventually eliminate its role entirely (Brookfield, 1992). Certain genes, despite their individual dispensibility, are highly conserved at the molecular level, leading to the suggestion that they may have some hidden positive selective value (Maier et al., 1993). Nevertheless, the apparent conflict between the prevalence of functional redundancy and the assumption of classical genetics that a mutation in a single gene should lead to a defined phenotype that will provide insight into the gene's function, has led to the proposal that a new paradigm is required for the understanding of how biological systems work (Tautz, 1992).

In considering morphogenetic processes, if we interpret the notion that forms result from differential gene activities as meaning that the presence of certain gene products at specific times and places helps channel the embryo along morphogenetic pathways defined by the available possibilities for physical change, then the conundrum of redundancy has a plausible solution. If segmentation, for example, were initially brought about in an evolutionary lineage by the coupling of a reaction-diffusion-based pattern forming mechanism with the differential regulation of cell-cell adhesion (Fig. 1I; 2B), this morphological outcome would be transmitted to the organism's offspring in a variable fashion, subject to fluctuations of temperature, pH, and chemistry, not only in the external environment, but in the internal metabolic milieu. Any molecular circuitry that protected the system against such fluctuations (Fig. 3B), would be advantageous to the sublineage that contained it. Such gene products or circuitry could have functions as different from one another as the various biochemical systems they help to buffer. A unique selective advantage would thus be associated with each of them. But as far as morphogenetic determination was concerned, they would be "redundant". Correspondingly, their targeted elimination might lead to small reductions in viability, but no "phenotype".

Waddington referred to genetic change that leads to increased resistance to diversion from specific developmental pathways as "canalizing selection" (Waddington, 1957), and Schmalhausen used the term "stabilizing selection" (Schmalhausen, 1949) for the same phenomenon. In contrast, Schmalhausen used "dynamic selection" to denote the selective advantage, under altered environmental conditions, that certain morphological variations have over a norm established over previous conditions of existence (Schmalhausen, 1949). If the only source of

morphological novelty considered is the classical Darwinian one of small, genetically-specified, random variations in the expression of morphological traits, then dynamic selection must be the driving force of phylogenetic change, with stabilizing selection playing a subsidiary role. When templating of morphogenetic alternatives by generic physical mechanisms is brought into the picture, however, stabilizing selection, or canalization, takes on much greater significance. But it follows from the previous discussion that a major consequence of stabilizing selection will be functional redundancy in genes involved in developmental regulation.

Conclusions

Darwin himself subscribed to the notion of an intimate connection between morphological evolution and embryonic development, and, indeed both processes were referred to as "evolution" in the scientific usage of the time (Richards, 1992). Attention to the relationship between these phenomena fell out of fashion as embryology and genetics drifted apart earlier in this century, and the latter, but not the former, became a central component of the neo-Darwinian synthesis. The embryologist August Weismann (1834–1914) validated the divergence between development and transmission genetics by emphasizing the fact that the germ line in most animals is set aside before any significant somatic morphogenesis has taken place (Weismann, 1893). If genes, which could mutate only in a random fashion, were assumed to determine all important organismal traits, and the criteria for gene selection were contingent on the reproductive success of adult forms, the relevance of embryogenesis to evolution was unclear, and easy to dismiss.

But the properties of multicellular aggregates, such as early embryos, go beyond those specified by the details of the genotype. The generic physical characteristics of small, viscoelastic, chemically active parcels of matter constitute one such set of properties, with inescapable relevance to tissue behavior. Furthermore, even in those species with sequestered germ lines, the conditions for the action of these physical processes are reinstituted in each generation, when fertilization and cleavage give rise to a new multicellular aggregate.

Since virtually every example of biological morphogenesis represents a sequence of rearrangements of chemically active soft matter, it follows that the set of possible morphologies is not inexhaustible. Indeed, as discussed above, position-dependent modulation of cell-cell adhesion in multicellular aggregates can potentially generate the major features of all body plans and organ forms. The capacity to produce hard tissues, which appeared several times during the course of evolution, introduced new levels of complexity, in that the corresponding lineages were thereby made resistant, in part, to the laws governing soft matter. It is notable, however, that during the development of modern animals, body plans and organ forms are generally established when the relevant primordia are soft. Development of skeletal structures occurs at later stages. Although this is no guarantee that evolution followed the same course, the fossil record suggests that animal forms were quite

elaborate even before the emergence of skeletal materials (Glaessner, 1984; Fedonkin, 1985; Conway Morris, 1993).

The rigid walls of plant cells would seem to preclude developmental mechanisms involving cell-cell rearrangement, which contributes to the liquid-like behavior of embryonic animal tissues (Steinberg, 1978; Steinberg and Poole, 1982; Armstrong 1989). In the cellular slime molds, however, which exhibit "animal-like" motile and developmental behaviors (Bonner, 1967), cell sorting-out and differential adhesion appear to contribute to morphogenesis prior to the consolidation of the cell wall (Bonner, 1971; Sternfeld, 1979; also see Nanjundiah and Saran, 1992 for a recent review). This suggests that morphogenetic differences between plants and animals may be partly the result of the different generic physical processes available to their respective tissues.

If morphogenesis is thus considered to be inherent to multicellular aggregates, it becomes useful to consider the role of morphogenetic regulatory genes as being the release and channeling of latent possibilities, rather than the producers of form. This would have been particularly evident in the early stages of morphological evolution when multicellular organisms would have contained many fewer genetic redundancies than they do now, however, much their forms might have resembled those of modern species. Under such circumstances small genetic alterations (of proteins involved in an adhesive interaction, or in a reaction-diffusion system, for example) would often have had wide-ranging effects on organismal or organ morphology. They could have influenced whether an organism developed segmentally, or not, with an internal cavity, or not, etc. As developmental pathways become increasingly overdetermined by stabilizing evolution, morphogenetic regulatory genes would have exerted subtler effects. These might have been realized as changes in the relative rates of different generic processes, leading to developmental heterochrony (DeBeer, 1940; Gould, 1977; McKinney, 1991). They could also have been reflected in position-dependent modulation of common biosynthetic activities by nonuniformly distributed regulatory factors (*e.g.*, HOM/*Hox* gene products, retinoid receptors), leading to subtly different identities of repetitively generated segments (Lewis, 1978; Akam et al., 1988) or skeletal elements (Morgan et al., 1992; Newman, 1988). In contemporary organisms, the phenotypes of the corresponding mutants or "knock outs" can be correspondingly subtle. In even more extensively canalized pathways the inactivation of "key" genes may have no effect at all on the morphological phenotype (Campos-Ortega and Knust, 1990; Rudnicki et al., 1992; Saga et al., 1992; Hulskamp et al., 1989; Irish et al., 1989).

A consequence of this perspective is that after an initial burst of morphological evolution, during which small genetic changes could drive large-scale transitions between physically-templated forms, the effect of mutations will be increasingly "Mendelian", in that alterations in single genes will often lead to small phenotypic changes, the relative frequency of which can be altered by natural or artificial selection. But in modern, highly canalized taxa, such changes would rarely extend beyond the perimeter of the species. The genetic variations that are the raw material of macroevolutionary change, in this view, are characteristic not of the modern organisms reflected upon by Darwin, but of an early stage of multicellular life.

These considerations address in a unified fashion a number of perennial and newly identified questions in developmental and evolutionary biology: why organisms have the particular organizational properties they do, why developmental pathways are so resistant to diversion, why phylogenetic innovations arose so abruptly and remained static for so long, and why functional redundancy among genes is so prevalent. The test of the view proposed here will be the coherence it may bring to results of continued investigation of the properties of embryos and ancestors.

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