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Epigenetic Mechanisms of Character Origination

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ABSTRACT The close mapping between genotype and morphological phenotype in many contemporary metazoans has led to the general notion that the evolution of organismal form is a direct consequence of evolving genetic programs. In contrast to this view, we propose that the present relationship between genes and form is a highly derived condition, a product of evolution rather than its precondition. Prior to the biochemical canalization of developmental pathways, and the stabilization of phenotypes, interaction of multicellular organisms with their physicochemical environments dictated a many-to-many mapping between genomes and forms. These forms would have been generated by epigenetic mechanisms: initially physical processes characteristic of condensed, chemically active materials, and later conditional, inductive interactions among the organism's constituent tissues. This concept, that epigenetic mechanisms are the generative agents of morphological character origination, helps to explain findings that are difficult to reconcile with the standard neo-Darwinian model, e.g., the burst of body plans in the early Cambrian, the origins of morphological innovation, homology, and rapid change of form. Our concept entails a new interpretation of the relationship between genes and biological form. *J. Exp. Zool.* (*Mol. Dev. Evol.*) *288:304–317, 2000.*

Evolutionary biology is currently the scene of debates around such topics as the tempo and mode of phenotypic evolution, the degree to which genetic change can result from selectively neutral mechanisms, and the universality of adaptation in accounting for complex traits. But in all the contending views the notion that an organism's morphological phenotype is determined by its genotype is taken for granted. This tenet is also essentially undisputed in developmental biology, which today is commonly characterized as the study of "genetic programs" for the generation of body plan and organ form. Here we explore the validity of this widely held notion, and suggest that an alternative way of looking at the causal relationship between genes and form can resolve some of the debates in evolutionary theory, as well as apparent paradoxes that have arisen with recent findings of extensive functional redundancy in developmental systems. In particular, we propose that the correlation of an organism's form with its genotype, rather than being a defining condition of morphological evolution, is a highly derived property. This implies that other causal determinants of biological morphogenesis have been active over the course of evolution, and that a theory of morphological evolution based on neo-Darwinian mechanisms alone must remain incomplete.

We set out from the observation that many organisms, particularly among the bacteria, protists, and fungi, but also among higher animals such as arthropods, molluscs, and vertebrates (as well as many plants), exhibit phenotypic polymorphism and morphological plasticity. Radically different forms occur in different settings or different phases of the life cycle. These distinct forms could represent independent adaptations, each realized by a separately evolved genetic subroutine. Alternatively, rather than being adaptive, morphological plasticity could reflect the influence of external physicochemical determinants on any material system. If the latter is the case in at least some instances in contemporary organisms, it is plausible that in earlier multicellular forms this externally-conditioned kind of morphological determination was even more

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prevalent. This is because ancient organisms undoubtedly exhibited less genetic redundancy, and metabolic integration and homeostasis, than modern organisms, and were thus more subject to external molding forces. Thus it is proposed that morphological variation in response to the environment is a primitive, physically-based property. This property is characteristic of all "soft matter" (deGennes '92), and "excitable media" (Mikhailov '90; Winfree '94) (see below), and would have been an inevitable feature of the viscoelastic cell aggregates that constituted the first multicellular organisms.

Examining the morphological plasticity of some modern organisms can provide insight into the flexible, environment-dependent relationship between genotype and form that still prevails in most of the living world. *Candida albicans*, for instance, a frequent fungal pathogen in humans, is able to switch among forms ranging from single budding cells, to threadlike hyphae, to strings of yeast-like cells plus long septated filaments, known as pseudohyphae (Braun and Johnson '97; Ishii, et al. '97). These and other considerations have led to the suggestion that *C. albicans* has no "default" morphology (Magee '97). Even in vertebrates the environment can play a decisive role in morphological development. For example, incubation temperature determines sex in reptiles in a species-dependent fashion—high temperatures produce males in lizards and crocodiles, but females in chelonians (Deeming and Ferguson '88). In mice the number of vertebrae can depend on the uterine environment: fertilized eggs of a strain with five lumbar vertebrae preferentially develop into embryos with six vertebrae when transferred into the uteri of a six vertebrae strain (McLaren and Michie, '58). Animals that undergo metamorphosis, such as echinoderms, tunicates, arthropods, and amphibians, also exhibit multiple morphological phenotypes, and metamorphosis can be influenced by environmental change as well as intrinsic timing mechanisms (Gilbert et al. '96).

The pervasiveness of plasticity and polymorphism suggests that the correspondence of a genotype to one morphological phenotype, as typically seen in higher animals, should be considered exceptional—a highly derived condition in which an "overdetermining" genetic circuitry ensures that changes of extrinsic or intrinsic variables have less impact on the morphological outcome. If modern-day organisms are Mendelian, in the sense that genotype and phenotype are inherited in close correlation, and for which morphological change is most typically dependent on genetic change, then the polymorphic metazoan ancestors we postulate would have constituted a pre-Mendelian world of living organisms, whose genotypes and morphological phenotypes were connected in only a loose fashion.

In this exploratory period of organismal evolution the mapping of a given genotype to a morphological phenotype would have been one-to-many, rather than one-to-one. The prototypes of modern forms, however phenotypically distinct, were probably totally or partially interconvertible at the generative level. Only later, with the evolution of genetic redundancies (Tautz '92; Picket and Meeks-Wagner '95; Wagner '96; Cooke, et al. '97; Nowak, et al. '97; Wilkins '97) and other mechanisms supporting reliability of developmental outcome (Rutherford and Lindquist '98), a closer linkage between genetic change and phenotypic change was established, with evolution under selective criteria favoring the maintenance of morphological phenotype in the face of environmental or metabolic variability. Organisms thus characterized by a closer mapping of genotype to phenotype, marked the transition from the pre-Mendelian to the Mendelian world.

This scenario of different phases in morphological evolution raises the possibility that the origination of organismal forms and characters, and their adaptive fine-tuning, are based on different mechanisms. Moreover, it points to an important conceptual gap in current evolutionary theory. Neo-Darwinism, in its present form, deals competently and successfully with the variation and adaptation of characters, but sidesteps the problem of their causal origin. Thus the emergence and organization of discrete morphological units still remains an open problem, recognized under the terms of "novelty"or "innovation"(Müller '90; Müller and Wagner '91).

The essence of the concept we will develop in the following pages is that epigenetic mechanisms, rather than genetic change, have been the major sources of morphological innovation in evolution. We do not use the term "epigenetic" to refer to DNA-related mechanisms of inheritance, such as methylation and chromatin assembly (see Jablonka and Lamb '95 for a review). The epigenetic mechanisms that we consider are conditional, non-programmed determinants of individual development, of which the most important are (1) interactions of cell metabolism with the physicochemical environment within and external to the organism, (2) interactions of tissue masses with the physical environment on the basis of physical laws inherent to

condensed materials, and (3) interactions among tissues themselves, according to an evolving set of rules. We suggest that different epigenetic processes have prevailed at different stages of morphological evolution, and that the forms and characters assumed by metazoan organisms originated in large part by the action of such processes.

A number of earlier authors have discussed the role of epigenetic factors in evolution. Some have argued for the importance of developmental constraints in influencing the direction of phenotypic change (Alberch '82; Maynard Smith, et al. '85; Stearns '86) or emphasized environmental effects on development (Johnston and Gottlieb '90). Other authors have pointed to the intrinsic dynamical structure of developmental systems in accounting for non-random variation of traits (Ho and Saunders '79; Kauffman '93; Goodwin '94). Our concept goes beyond these suggestions in postulating that the processes by which morphological characters are determined are different at different phases of evolution, with genetic integration taking on a more prominent role after a character is established. In particular, our view involves the recognition that forms and characters produced by epigenetic factors can serve as templates for the accumulation of overdetermining genetic mechanisms. As a result, the action of the originating epigenetic factors may be obscured or even superseded in modern developmental systems.

The relationship between genes and biological form is not simple, and the standard notion of the "genetic program" is increasingly seen as problematic (Oyama '85; Nijhout '90; Müller and Wagner '91; Bolker and Raff '96; Neumann-Held '98). We propose a revised interpretation of that relationship: with regard to the origin of morphology, we take the physical nature of living organisms to be their most salient property. This implies that epigenetic processes, which are contingent and conditional, are the motive forces in the evolution of biological form. As evolution proceeds, genetic change that favors maintenance of morphological phenotype in the face of environmental or metabolic variability co-opts the morphological outcomes of epigenetic processes, resulting in the heritable association of particular forms with particular genealogical lineages.

We note that the notion of "evolvability"—the inherent potential of certain lineages to change during the course of evolution—is interpreted in an entirely different fashion in light of the ideas presented here than it has been in other recent discussions (Gerhart and Kirschner '97; Kirschner and Gerhart '98). For us evolvability represents the continued efficacy of epigenetic processes in a lineage—some of them quite ancient, and some of more recent origin—and as such is tied to the primitive morphogenetic plasticity hypothesized above. Genetic evolution, particularly of the cooptative kind, will tend to suppress such evolvability and buffer the development of form. This contrasts with the view that evolvability is a product of advanced evolution, achieved by the emergence of new genetic mechanisms that favor plasticity.

EPIGENESIS IN A "PRE-MENDELIAN" WORLD

The physics of tissue masses and the origin of body plans

Multicellular organisms first arose more than 600 million years ago (Conway Morris '93). By approximately 540 million years ago, at the end of the Cambrian explosion, virtually all the "bauplans" or body types seen in modern organisms already existed (Whittington '85; Conway Morris '89; Briggs, et al. '92). The original multicellular forms were established with cells that were metabolically and structurally sophisticated—the first eukaryotic cells appeared at least a billion years earlier (Knoll '92). Although many, if not most, of the genes present in modern multicellular organisms were already in place, encoding corresponding proteins with well-defined roles in unicellular structure and function, these genes and proteins had not been selected for the construction of multicellular characters.

The most ancient multicellular forms must have been simple cell aggregates that arose by adhesion of originally free-living cells, or by the failure of the same to separate after mitosis (Bonner '98). The precise chemical or physical nature of the adhesive interaction would have been unimportant, as long as it served to keep the organism's cells from dispersing. Indeed, the advent of a cell-cell adhesion mechanism early in the history of multicellular life, although certainly dependent of the preexistence of particular gene products, need not have required additional gene sequence change. For example, some modern cell surface proteins, such as the cadherins, mediate cell attachment only in the presence of calcium ions (Takeichi '91). Protein chemists are well aware that many proteins that perform no adhesive function at all exhibit different degrees of "stickiness" under different ionic conditions. It is thus plausible that a protein on the surface of an ancient unicellular

eukaryote could have acquired a new function—adhesion—by virtue of a simple change in the ionic content of the organism's aqueous environment, leading to simple multicellular forms by fiat (Kazmierczak and Degens '86).

While the appearance of primitive multicellular forms in the fossil record may have thus been a relatively straightforward matter (Bonner '98), not dependent on the evolution of any complex developmental machinery, the "heritability" of the multicellular state would have depended either on the persistence of the new external conditions, or on the evolution of adhesion proteins that were less dependent on context. The earliest multicellular organisms, however, were unlikely to have generated their forms using the baroque, hierarchical, molecular machinery that guides morphogenesis in modern organisms (Nüsslein-Vollhard '96). Rather, the existence of a simple mechanism of adhesion, whereby cells could remain attached to one another after they divided, would have been sufficient to establish multicellularity.

Compartmentalization, tissue multilayering, and segmentation

Once one or several adhesive mechanisms were in place, other more complex morphological consequences could have inevitably followed, simply by virtue of variations in cell adhesivity brought about by random processes like metabolic noise, and by the way in which the relevant physical laws act on such heterogeneous cell aggregates. Cells with different amounts of adhesion molecules on their surfaces, for example, tend to sort out into islands of more cohesive cells within lakes composed of their less cohesive neighbors. Eventually, by random cell movement, the islands coalesce and an interface is established, across which cells will not intermix (Steinberg and Takeichi '94; Steinberg '98). What is observed is similar to what happens when two immiscible liquids, such as oil and water, are poured into the same container. An important feature of this mechanism is that the final morphological outcome is independent of the initial conditions—in effect it is goal-directed. Thus, when several differentially adhesive cell populations arise within the same tissue mass, multilayered structures can form automatically, comprising distinct compartments (Crick and Lawrence '75; Garcia-Bellido et al. '76) (Fig. 1A). Indeed, two of the five major types of gastrulation seen in modern metazoans, *epiboly and involution* (and possibly a third, *delamination)* (Fig. 1C), could have originated as simple consequences of differential adhesion (Newman '94).

Thus, somewhat counterintuitively, lax regulation of the abundance of adhesion proteins, in conjunction with thermodynamic processes, can lead rather directly to novel, multilayered organismal forms. Furthermore, if variations in metabolic or biosynthetic activity, rather than being purely random across the tissue mass, affected cell-cell adhesion in a temporally or spatially periodic fashion, then *compartmentalization*—the establishment of boundaries of immiscibility—takes the form of *segmentation* (Newman '93) (Fig. 1D). Moreover, the generation of periodicities is all but inevitable in the complex, excitable media represented by even the simplest aggregates of cells.

Excitable media are materials that actively respond to their environment, mechanically, chemically, or electrically. Nonliving examples have been well studied (Gerhardt, et al. '90; Mikhailov '90; Starmer, et al. '93; Winfree '94). Aggregates of living cells, embodying metabolic and genetic networks responsive to the external environment, and containing positive and negative feedback loops and diffusible components, will have tended spontaneously to develop chemical oscillations (Goldbeter '95) and spatial periodicities (Turing '52; Boissonade et al. '94). From such biochemical periodicities it is only a few steps to segmental tissue organization (Palmeirim, et al. '97; Pourquié '98), which is therefore likely to have arisen numerous times in the history of life (Newman '93).

Cell polarity and lumen formation

The first multicellular organisms plausibly were composed of cells with a uniform, or random, distribution of adhesive molecules on their surfaces. Many modern cell types, in contrast, are polarized, capable of allocating different molecular species to their apical and basolateral regions (Rodriguez-Boulan and Nelson '93). The targeting of adhesive molecules, or anti-adhesive molecules, to specific regions of the cell surface has dramatic consequences. A tissue mass consisting of motile epithelioid cells that are non-adhesive over portions of their surfaces would readily develop cavities or lumens. If such spaces were to come to adjoin one another, as a result of random cell movement, they would readily fuse (Fig. 1B). Lumen formation may therefore have originated as a simple consequence of differential adhesion in cells that express adhesive properties in a polarized fashion. The formation of lumens in masses of mammary carcinoma cells by the forced, polar expression of the *met* oncogene (Tsarfaty et al. '92) is a model for this morphological innovation in a contemporary system.

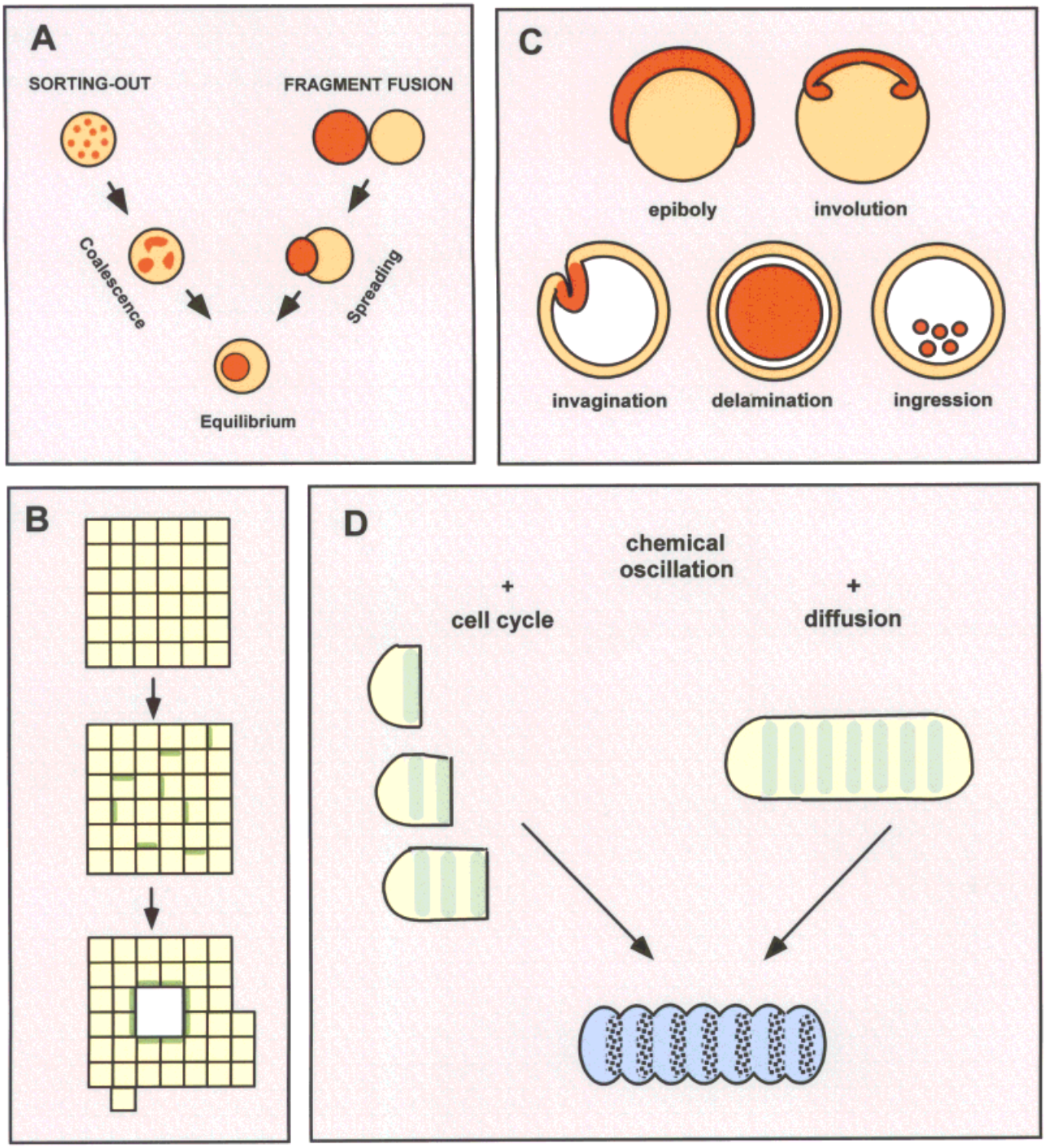

Figure 1.

Significantly, the first morphologically complex multicellular organisms, represented by the Vendian fossil deposits dating from as early as 700 million years ago, appear to have been flat, often segmented, but apparently solid-bodied creatures (Seilacher '92; Conway Morris '93). Among modern phyla the coelenterates, such as hydra, are forms with a single lumen; echinoderms (e.g., starfish) and vertebrates have both a digestive tube and a surrounding body cavity. It is thought to have taken up to 100 million years after the appearance of the Vendian fauna for organisms

to develop distinct body cavities, although recent evidence suggests that this may have occurred more rapidly (Seilacher et al. '98). Once these triploblastic forms arose, all the modern body plans burst onto the scene in short order.

It is interesting to consider the possibility that the advent of polarized cells may have provided the physical basis for the rapid profusion of body types during the Cambrian explosion. Depending on the taxon, cell polarity could have arisen before or after the evolutionary event that led to multicellularity. In either case, lumen or cavity formation would have been an inevitable physical consequence of the conjunction of these two properties. Two of the major types of gastrulation—*invagination* and *ingression* (Fig. 1C)—whatever the mechanisms of their realization in modern animal phyla, could have originated in ancient organisms by the actions of differential adhesion in establishing multiple tissue layers in conjunction with lumens in cell aggregates (Newman '94).

The combined effects of the various physical properties that were generic to the earliest multicellular aggregates considered as chemically excitable, viscoelastic soft matter, will thus have ensured the production of a profusion of multilayered, hollow, segmented forms—a pre-Mendelian world of fully or partially interconvertible prototypes for the genetically routinized body plans to come. While not every physically attainable multicellular form would necessarily prosper, many strikingly different kinds would. Moreover, the surviving morphotypes define, in a real sense, their own ecological niches, rather than representing merely adaptations to pre-existing ones. A novel implication of this interpretation of the burst of forms during the early history of metazoan life is that the disparate organismal forms would have been achieved with no requirement for competition or differential fitness. Since function would follow form, rather than the other way around, the pre-Mendelian world would thus also have been, in this sense alone, a "pre-Darwinian" one.

Fig. 1. Generic processes in tissue morphogenesis. **A**: Schematic representation of the behavior of intermixed cells and corresponding tissue fragments in the case where the two cell populations are differentially adhesive. The cell mixture will sort out as the more adhesive cells establish more stable bonds with one another than with cells of the other population. Random motion leads to the formation of cohesive islands of these cells, and these will ultimately coalesce into a separate tissue phase, or compartment. The equilibrium configuration of the cell mixture is identical to that which would be formed by fusion and spreading of fragments of tissue consisting of the same differentially adhesive cell populations. **B**: Schematic view of formation of a lumen or internal cavity by differential adhesion in an epithelioid tissue consisting of polarized cells. In the original state (top) the cells are uniformly adhesive, and make contacts around their entire peripheries. Upon expression of an anti-adhesive protein (green) in a polarized fashion in a random subpopulation of cells (center), and random movement of the cells throughout the mass, bonds between adhesive surfaces are energetically favored over those between adhesive and nonadhesive surfaces, resulting in lumen formation (bottom). **C**: Schematic cross-sectional views of the five main types of gastrulation. In each case a new population of cells differentiates from a solid or hollow embryo and assumes a position that would be attained by a similarly situated differentially adhesive population. **D**: Schematic representation of two modes of tissue segmentation that can arise when the tissue's cells contain a biochemical circuit that generates a chemical oscillation or "molecular clock," and the oscillating species directly or indirectly regulates the strength or specificity of cell adhesivity. In the mechanism shown on the left, the periodic change in cell adhesivity occurs in a growth zone in which the cell cycle has a different period from the regulatory oscillator; as a result, bands of tissue are sequentially generated with alternating cohesive properties. In the mechanism shown on the right, one or more of the biochemical species can diffuse, leading to a set of standing waves of concentration of the regulatory molecule by a reaction-diffusion mechanism. This leads to the simultaneous formation of bands of tissue with alternating cohesive properties. See Newman, '93 for additional details. (A, with changes, from Steinberg, '98; B after Newman and Tomasek, '96)

EPIGENESIS IN A MENDELIAN WORLD

Source of innovation and homology

Once major body plans were established, selection for biochemical integration, which promoted physiological homeostasis and developmental reliability, stabilized the relationship between genotype and ecological setting referred to as fitness or adaptedness. This increasingly unique matching between genotype and phenotype led ultimately to Mendelian heritability. Morphological innovation leading to diversification at the subphylum level was to follow. While the standard picture holds that this was the virtually exclusive result of incremental selection of minor, random, phenotypic variants, we suggest that epigenesis was also an important driving force in these later events.

As a consequence of compartmentalization, organisms came to contain differentiated subpopulations of cells with the potential to perform specialized functions. The biochemically divergent tissues formed from such cells provided components of one another's environment, and as the forms produced began to depend on their interactions, *embryonic induction* came into existence. The *conditionality* of tissue interactions, along

with residual generic morphogenetic properties, guaranteed that the resulting systems retained a significant degree of "play." Variations in the natural developmental environment, like experimental perturbation (Hall '84; Müller '89), can divert even highly evolved systems into alternative pathways, with physical factors continuing to play an influential role. Even in the developmental systems represented by modern-day metazoa, by no means are all components strictly determined by the genome (see below). Rather, such systems are characterized by an interplay between epigenetic and genetic control, which generates reliable phenotypic outcomes. As a consequence of the continued conditional nature of evolved development, evolutionary modifications that affect one part of a system can have strong effects on other parts, leading to "unexpected" phenotypic innovations.

The continuity of physical influences

Earlier we indicated how the generic physical properties of tissues would have strongly influenced the array of forms generated in early organismic evolution. Although the role of these physical processes in the formation of body plans must have receded as more developmental interactions and the associated biochemical inertia set in, physical principles, and biomechanical factors in particular, remained active in secondary developmental fields and had important consequences for the further evolvability of phenotypic design.

Evolved morphogenesis is largely a matter of molding clusters of dividing cells into physical shapes. Layers, sheaths, tubes, rods, spheres, etc. are formed by aggregates of cells, mobilizing a wide range of biomechanical forces that result from the different properties of different cell types and their extracellular products (Fig. 2A-C). Once these macro-shapes have formed, their macroproperties in turn become important parameters for further development, not only creating geometric templates and barriers, but also controlling gene activity. These higher level physical factors become a part of the developmental program that is not explicitly specified in any inherited code of information. Their existence, however, determines what may result from a developmental system, both in a constraining and a generative manner.

As an illustration we consider the vertebrate limb. The origin and evolution of limbs is largely a consequence of evolving an internal skeleton. Skeletogenesis is based on the generic capacity of mesenchymal cells to adhere and condense, and produce cartilage matrix. During limb development this sequence of events is constrained by the spatial confinements of the limb bud and modulated genetically through differential cell adhesion (Yokouchi et al. '95; Newman '96). In developing mesenchyme the presence of diffusible, positively autoregulatory effectors of extracellular matrix production (such as transforming growth factor-beta) along with diffusible inhibitory factors, can lead to spatial periodicities in the conditions required for chondrogenesis (Newman and Frisch '79; Newman '88; Leonard et al. '91; Newman '96; Miura and Shiota, 2000a,b). Spatial self-organization of the limb bud mesenchyme thus leads to a basic pattern of repeating

Fig. 2. Epigenetic mechanisms of tissue morphogenesis and organogenesis. **A**: Schematic representation of major modes of epithelial morphogenesis resulting from extrinsic alteration of cell parameters. In a, a pattern formation mechanism (e.g., a reaction-diffusion system) is activated in a flat epithelial sheet (green), possibly mediated by a subjacent mesenchymal layer (brown), and marks a subset of cells to undergo alteration of one or more "potential functions" (e.g., adhesive strength, cytoskeletal tension.). In b–e, resulting epithelial morphologies are indicated. A placode, b, will form if the lateral regions of the epithelial cells become more adhesive than the apical and basal regions. An evagination, b, as in a developing intestinal villus, or an invagination, d, as in a developing hair or feather (Chuong and Widelitz '98) will form if the change in cell potential gives rise to a "bending moment" (Newman '98) that destabilizes the flat configuration. Progressive cycles of patterning and invagination will give rise to a branched tubular structure, e, as in salivary gland morphogenesis (Kashimata and Gresik '96). **B**: Schematic representation of mesenchymal condensation, as occurs during skeletal morphogenesis and many other developmental processes. Such condensations can be initiated by local patches of elevated production of extracellular matrix (ECM) molecules, and consolidated by cell-cell adhesion. **C**: Morphogenesis of connective tissue elements, such as cartilage rods and nodules, occurs by the regulation of the pattern of mesenchymal condensation formation. One way that this can occur is by the interplay of a positively autoregulatory diffusible activator of ECM production, such as TGF-beta (red arrows), with a diffusible inhibitor of its activity (violet arrows). In the absence of the inhibitor (top) resulting cartilage forms as an amorphous mass; in its presence, patterns of well-spaced nodules and rods can form as centers of activation become surrounded by domains of inhibition. **D**: Origin of the "fibular crest" in archosauran hindlimbs by mechanical regulation of mesenchymal morphogenesis. Progressive evolutionary reduction of the fibula increases the mechanical load on the connective tissue between the tibia (yellow) and the fibula (brown), exerted by the pulling action of the iliofibularis muscle (red). A stress-dependent cartilage (blue) forms in response and becomes later incorporated into the ossifying tibia to form a prominent crest (stippled), a homologue shared by theropod dinosaurs and carinate birds. This tight fixation of the proximal fibula permits its further distal reduction in avian limbs. (B and C after Newman, '96; D adapted from Müller and Streicher, '89).

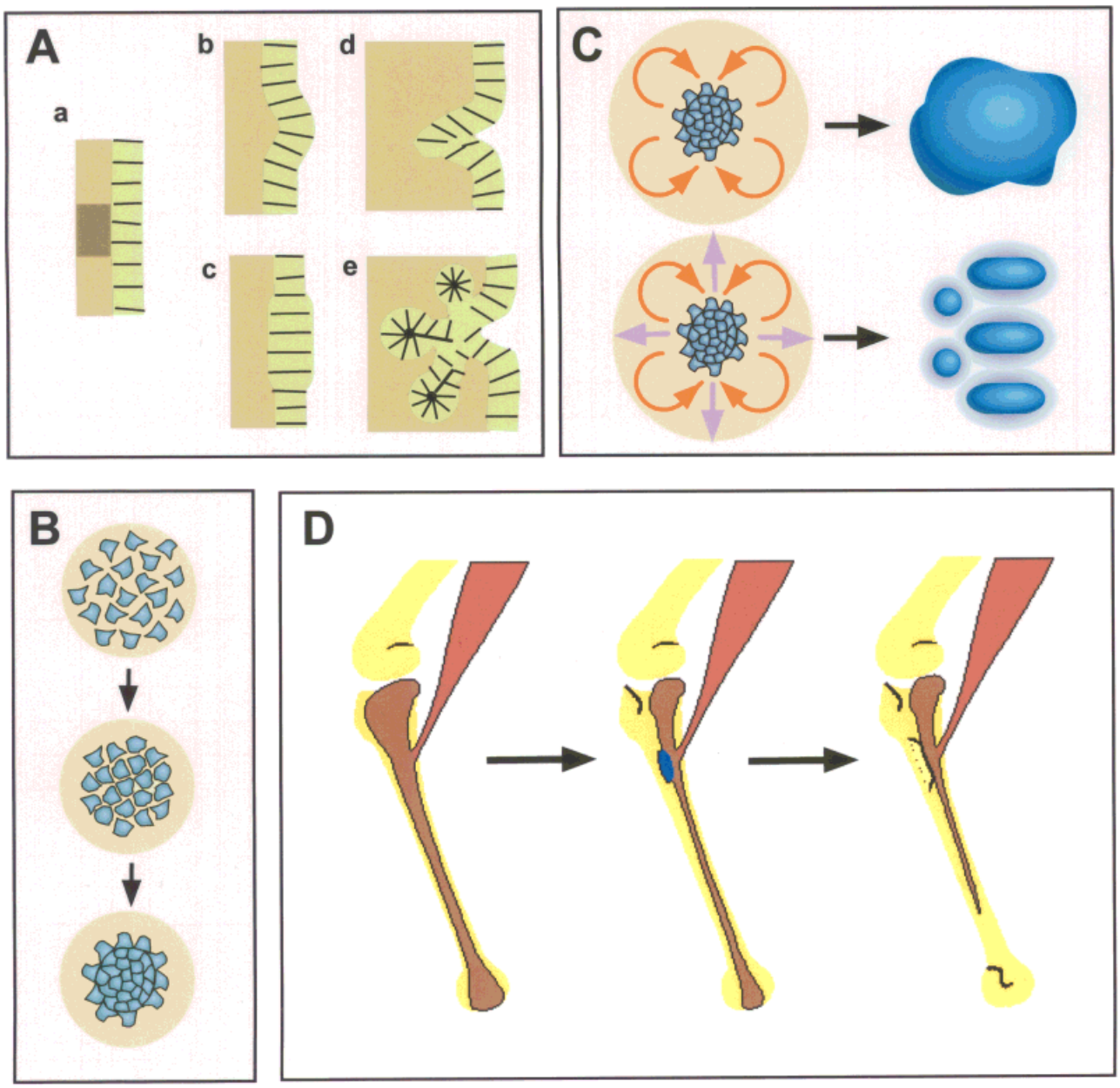

Figure 2.

skeletal elements. The evolution of the vertebrate limb can be viewed as the history of molecular and genetic modulation of developmental mechanisms (Shubin et al. '97) that are fundamentally generic and physical. Later, additional physical factors become important as muscle contractions and embryonic movements begin to influence bone and joint formation (Drachman and Sokoloff '66; Persson '83; Amprino '85; Hall '86), muscle and tendon differentiation (Scott et al. '87; Giori et al. '93), and consequently innervation (Dahm and Landmesser '91), blood vessel patterns, etc. This means that the generic properties of limb tissues contribute not merely to skeletogenesis but eventually influence downstream development. Physical factors thus continue to be of decisive importance even in contemporary ontogenies.

Innovation at the phenotypic level

As a result of the increasingly homeostatic nature of evolved development, changes affecting one component of a system could now have strong ef-

fects on associated components. The existence of thresholds in developmental processes, and the systemic consequences of modified morphogenesis, can create unexpected by-products, which may appear as phenotypic innovations at the subphylum level (Müller '90; Müller and Wagner '91).

Connective tissue and tendons, for instance, have the capacity to react to biomechanical stimuli by forming cartilage and bone. Such skeletal elements, known as cartilaginous or ossified sesamoids, can arise as a consequence of changes of bone proportions, for example: the altered stresses on embryonic connective tissue and tendon insertions generate novel sesamoids. In the avian hindlimb four such movement-dependent sesamoids form during the course of normal development. They remain fully dependent on the biomechanical stimuli of embryonic movement, as shown by paralysis experiments that inhibit their formation (Wu '96). Later, during ossification, these skeletal elements become incorporated into the longbones of the limb. Evolutionary changes of bone proportion (Streicher and Müller '92) will generate similar changes in embryonic biomechanics, and the resulting skeletal elements appear as novel characters of avian bones, such as the supratendinal bridge, the cnemial process, or the fibular crest of the tibiotarsus (Müller and Streicher '89) (Fig. 2D). They all represent significant changes of bone morphology, yet none of these characters will have arisen as a direct result of a mutation or "new genes" for that specific character. Rather they arise as side effects of mutations affecting other characters, such as the size or the growth rate of the tibia. The novelties that result from the consequently altered biomechanical conditions will then become incorporated into the bauplan of the limb. In addition, the general stress dependency of the skeletogenic system further modulates the external shape and inner architecture of bones during postnatal activity (Carter and Orr '92).

In the highly advanced developmental systems of modern-day vertebrates such epigenetic side effects probably account only for relatively minor character innovations. But it is plausible that the entire endoskeleton of vertebrates arose in a similar fashion. In vitro and in vivo studies demonstrate that cells, and connective tissue cells in particular, arrange themselves along stress fields (Harris et al. '80; Bard '90). Moreover, cartilage matrix secretion is an autonomous property of mesenchymal cells, highly dependent on cell number and density (Cottrill et al. '87) and compression (Vogel and Koob '89; Robbins et al. '97). Therefore it is likely that any mesenchymal tissue mass above a certain threshold size, such as the embryonic body axis or lateral outgrowths from it, may have automatically begun to generate dense cores of matrix-secreting cell arrays along the stress fields generated by passive and active movement, thus stabilizing large mesenchymal cell aggregates and allowing the further increase of body size. The dynamic interaction between diffusible cytokines promoting and inhibiting the expansion of these aggregates, which themselves can be induced by mechanical loading (Klein-Nulend et al. '95), will have readily led to their periodic arrangement (Newman and Frisch '79; Newman '84, '96), giving rise to the vertebrae, ribs, digits, etc., of the modern endoskeleton.

While the evolution of biochemical circuitry and developmental control mechanisms would have subsequently fixed new traits that arose in ways such as described above, the strength of such fixation can be variable. The susceptibility of movement-dependent sesamoids to paralysis, for example, differs significantly (Wu '96). Thus it appears that the generation and the fixation of novelty are quite distinct processes, governed by different mechanisms (Müller and Wagner '91).

From homoplasy to homology

We have argued that epigenesis is a primary factor directing morphological evolution, even in evolved developmental systems. In particular, we have suggested that structural innovations were probably largely epigenetic in their origin. Although the population-level establishment of any morphological innovation will depend on the ecological conditions under which its carriers live (Liem '90; Galis and Drucker '96), innovations initially originate as "pure" consequences of ubiquitous material and developmental propensities. Therefore generic processes can lead to similar forms in unrelated organismal lineages, manifested as the characteristic "homoplasies" of morphological evolution (Wake '91; Sanderson and Hufford '96; Moore and Willmer '97). However, another characteristic of advanced morphological evolution was to prove crucial, namely the establishment of heritable anatomical units. This principle of organismal design, commonly referred to as homology, is central for any conceptual understanding of morphological evolution (Hall '94). The question thus arises of a causal relationship between homoplasy and homology.

We propose the following scenario: As "Mendelian" organisms with increased matching between

genotype and phenotype began to emerge, development originally based on generic physical tissue properties was stabilized, and specific outcomes of morphogenetic processes became templates for the organization of newly evolving and integrated biochemical circuitry. This led to developmental individualization and modular building units (Wagner '89, '95). But as these modules became functionally integrated and fixed at the bauplan level of a lineage, they in turn assumed a specific constructional identity at that level, becoming the elements of macroscopic design referred to as homologues. This morphological identity eventually transcends all processes involved in the ontogeny of an individual homologue, be they genetic, cellular, biochemical, or physical, since these can change over the course of evolution (Wagner '89; Wray and Raff '91; Hall '94; Bolker and Raff '96). Thus the stabilized macro-patterns (homologues) became more decisive for the further path of morphological evolution than the generic conditions on which they were initially based.

This means that although homologues may first arise by the same epigenetic processes that produce homoplasies, they eventually become independent of their underlying molecular, epigenetic, and generic constituents and increasingly play an organizational role in morphological evolution. They take on a life of their own and are thus inherited as structural units of morphological organization, not tied to any particular generative process. Homoplasies reflect the origin of morphological innovation in the generic material properties of tissues—they are an echo of the pre-Mendelian world. Homologues, in contrast, act as formal "attractors" of design, around which more design is added (Müller and Newman '99).

GENES AND FORM: A REINTERPRETATION

We propose that a synthetic, causal understanding of both development and evolution of morphology can be achieved by relinquishing a gene-centered view of these processes. This is not to say that programmed gene expression plays an unimportant role during embryogenesis, or that random genetic change is not a major factor of evolution. But we argue, in agreement with some earlier writers (Ho and Saunders '79; Oyama '85; Seilacher '91; Goodwin '94), that these factors are not explanatory of morphology in either of these settings. What replaces gene sequence variation and gene expression as morphological determinants in our framework are epigenetic processes: initially the physics of condensed, excitable media represented by primitive cell aggregates, and later conditional responses of tissues to each other, as well as to external forces. These determinants are considered to have set out the original morphological templates during the evolution of bodies and organs, and to have remained, to varying extents, effective causal factors in all modern multicellular organisms.

We emphasize that the indirect relationship of genes to form, which we postulate for tissue morphogenesis, is analogous to what is generally accepted to constitute this relationship in the most fundamental role of genes: protein synthesis. Here genes also influence the realization of form without being its determinants. The three dimensional, folded structure of a protein—its biologically functional morphology—is defined by interactions of the polypeptide chain within itself and with its external environment. The typical functional form of a protein is identical to that decreed by the thermodynamics of spontaneous processes. Correspondingly, the universe of protein secondary structures and folded motifs in existing organisms is limited to a relatively small number of forms (perhaps 1,000) out of an astronomically large number of potential random compact structures (Chothia '92; Li et al. '96). Although the folding that takes place in the cytoplasmic environment of the modern cell is not always thermodynamically spontaneous—energy-dependent chaperoning processes are frequently employed (Beissinger and Buchner '98)—evolution has clearly used the spontaneously achieved morphologies as templates for the accumulation of sophisticated reinforcing mechanisms.

Just as an understanding of the set of preferred protein motifs and the morphologies of particular proteins depends on an appreciation of the originating role of physical mechanisms, we contend that an understanding of the forms assumed by metazoan organisms requires knowledge of the generative epigenetic processes that originally (in evolutionary history) produced those forms. Morphological development in ancient or modern metazoans was and is dependent on genetically-specified biochemical constituents acting in the context of dynamic material systems with characteristic generic properties. As long as these generic properties dominated, the genes were merely suppliers of building blocks and catalysts, with little direct influence on the architectural outcome. But genetic evolution is highly suited for enhanc-

ing the reliability of generation of "generically templated" forms. Standard modalities of gene evolution, such as promoter duplication and diversification (Goto et al. '89; Small et al. '91), metabolic integration, and functional redundancy (Wilkins '97) can add parallel routes to the same endpoint (Newman '94). Eventually, some of the parallel routes may come to predominate, and constitute what has the appearance of a "genetic program," although (strangely, from the point of view of the computer metaphor) the physical outcome of the program's execution actually preexists the programmed "instructions." Thus physical morphogenesis would become secondarily captured and routinized by genetic circuitry, possibly involving mechanisms such as genetic assimilation (Waddington '61).

The "unit character" notion was considered problematic and dismissed early on by such pioneers as Johannsen and Morgan (see Dunn '65 for a discussion). A number of more recent writers have continued to point out the inadequacy of a view of genes that takes them individually to correspond to particular complex traits, or even collectively to constitute programs for the production of such traits (Oyama '85; Nijhout '90; Neumann-Held '98) (although this has not prevented some latter day commentators from writing about genes for stripes, tails, or fingers). As application of more precise methods in developmental and evolutionary biology provides increasing evidence that there is no necessary relation between genetic and morphological change (e.g., Atchley, Newman, and Cowley '88; Meyer et al. '90; Sturmbauer and Meyer '92; Bruna, Fisher, and Case '96), the need for a new synthesis along these lines becomes more compelling. What has been missing from previous discussions is any positive account of what (if not genes per se) causes metazoan organisms to take on the forms they do in the course of development and evolution. We propose that epigenetic processes play this positive role, either (to use Aristotle's famous distinction) as "efficient causes" early in evolution, and to a more limited extent in contemporary organisms, and as "formal causes" (i.e., templates) as evolution progresses and new ways are invented to achieve the same morphological ends. Indeed, evolution can be considered an engine for turning efficient causes into formal causes.

CONCLUSIONS

The formal framework of neo-Darwinian theory requires morphological characters to be given, and, therefore, does not constitute a theory of how they arise. We have here proposed a conceptual framework for the origination of morphological characters and their co-optation by the genome. We summarize our position in the following points:

1) The origin of form and characters is based on epigenetic principles acting both in the pre-Mendelian and the Mendelian world

- The earliest epigenetic mechanisms to influence biological form were the physics of chemically active condensed materials, which include primitive cell masses, resulting in a delimited, and essentially exhaustive, array of body plans and organ forms—segmented, hollow, multilayered, and branched structures.
- As a consequence of the biochemical and genetic integration of interactions, development increasingly takes place in a Mendelian arena in which genotype and morphological phenotype become more closely matched. Development also becomes susceptible to Darwinian modification leading to the exploration of the residual morphogenetic "play" remaining in multicellular systems. In particular, physical properties and threshold effects of the developmental systems under modification generate morphogenetic by-products that become the kernels of morphological innovations, which elaborate on a smaller scale the major morphological themes of the earlier phase.

2) The epigenetic concept addresses a number of open problems in evolutionary theory, such as the origin of body plans, morphological innovation, and homology.

- If epigenesis can account for the origins of bauplans and morphological innovation, competition among marginally different forms for adaptive advantage is not a sine-qua-non of morphological change. Darwinian adaptation-driven evolution can therefore be considered to be a limiting case of the epigenetic model. Selection, in this view, functions to release and consolidate inherent developmental potential, rather than guiding morphological evolution directly.
- Homology, the principle of morphological organization, is a consequence of the interplay between generic, morphogenetic templates and evolving, stabilizing biochemical circuitry. Fixed at the bauplan level, their molecular and developmental bases free to drift, homologues persevere and become attractors of morphological design.

3) The epigenetic concept entails a new interpretation of the relationship between genes and biological form

- The relationship between genotype and phenotype in the earliest metazoan organisms is hypothesized to have been different from that in modern organisms. The present relationship between genes and form is a derived condition, a product of evolution rather than its precondition.
- Evolvability, in general, represents the carryover of epigenetic determination from an earlier epoch of even greater morphogenetic plasticity, rather than the evolution of sophisticated genetic mechanisms selected to undermine rigid genetic determination.
- Genetic change is required for evolution to progress, but with respect to morphology it mainly plays a consolidating role, rather than an innovating one. Physically determined morphogenesis becomes secondarily captured and routinized by genetic circuitry that thus serves to channel and reinforce epigenetic propensities.

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