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Perspective

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ABSTRACT **Evolvability is an organism's capacity to generate heritable phenotypic variation. Metazoan evolution is marked by great morphological and physiological diversification, although the core genetic, cell biological, and developmental processes are largely conserved. Metazoan diversification has entailed the evolution of various regulatory processes controlling the time, place, and conditions of use of the conserved core processes. These regulatory processes, and certain of the core processes, have special properties relevant to evolutionary change. The properties of versatile protein elements, weak linkage, compartmentation, redundancy, and exploratory behavior reduce the interdependence of components and confer robustness and flexibility on processes during embryonic development and in adult physiology. They also confer evolvability on the organism by reducing constraints on change and allowing the accumulation of nonlethal variation. Evolvability may have been generally selected in the course of selection for robust, flexible processes suitable for complex development and physiology and specifically selected in lineages undergoing repeated radiations.**

Darwin based his origin of species theory on heritable variation and natural selection, although he conceded that "our ignorance of the laws of variation is profound" (1). Although much of the mystery of heredity and genetic variation has been dispelled by Mendelian genetics and the modern synthesis, the relationship of genetic variation to selectable phenotypic variation is far from understood (2). The consequences of mutation for phenotypic change are conditioned by the properties of the cellular, developmental, and physiological processes of the organism, namely, by many aspects of the phenotype itself. We may expect that many of these processes constrain variation, making much of it maladaptive. Nevertheless, we may ask whether certain properties of these processes bias the kind and amount of phenotypic variation produced in response to random mutation, such that more favorable and nonlethal kinds of variation are available on which natural selection can act.

The capacity of a lineage to evolve has been termed its evolvability, also called evolutionary adaptability. By evolvability, we mean the capacity to generate heritable, selectable phenotypic variation. This capacity may have two components: (*i*) to reduce the potential lethality of mutations and (*ii*) to reduce the number of mutations needed to produce phenotypically novel traits. We can ask whether modern metazoa of highly diversified phyla, have cellular and developmental mechanisms with characteristics of evolvability and whether this evolvability is under selection and has itself evolved.

The concept of evolvability was formulated in the past by several evolutionary biologists drawing from morphological examples such as limb, jaw, or tooth diversification (3–5), and the possible evolution of evolvability has been discussed (5–7). Evolvability also was formulated in theoretical models by several authors (7–9). Though artificial, these models confirm in principle that rules for generating phenotypic variation can affect the evolvability of a system. We will address evolvability at the molecular, cellular, and developmental levels with the conviction that it is more clearly demonstrable at these levels than at the level of morphology.

It is difficult to evaluate how the particular characteristics of cellular, developmental, and physiological mechanisms affect the quantity and quality of phenotypic variation after genetic change and hence affect evolvability. To understand the consequence of mutation for a protein's activity, one needs to understand the interactions of that protein with many other cell components. A current view is that conserved core processes constrain phenotypic variation, acting as a barrier to evolution (4, 6). Many core processes are conserved throughout metazoa (e.g., many signaling pathways and genetic regulatory circuits), others throughout eukaryotes (e.g., the cytoskeleton and cdk/cyclin-based cell cycle, and yet others throughout all life forms (e.g., metabolism and replication). It is natural to assume that highly conserved mechanisms are optimized after repeated selections or are "frozen accidents" (because natural selection works with the best available at the time, not the best possible) that are now extensively embedded in other mechanisms. By either assumption, the process would be highly constrained, and most changes would be detrimental, i.e., lethal. Much has been made of constraint in recent discussions of evolution (4, 6, 10). Constraint results from functional interactions of proteins with each other, other cell components, and environmental agents. The greater the number and exactness of a protein's requirements for function, the fewer the possibilities for changes of its amino acid residues by mutation. For example, if residues reside in a binding site for ligands or substrates or other proteins, they would be constrained to change. However, there is no reason to assume that constraint alone ensures evolutionary retention (survival) of a process. Even constrained processes must be conserved because of repeated positive selection (11)—the view we will espouse in this essay.

The exact nature of embedment and selection in the conservation of core processes will depend on the ecological history of a lineage of organisms. Metazoa have undergone rapid and diverse phenotypic change, particularly in morphology, tissue organization, development, and physiology, that has entailed an extensive elaboration of cell–cell communication. By contrast, eubacteria have undergone limited morphological change but have instead achieved extensive biochemical diversification. Bacteria are microscopic, asexual, ubiquitous, and slowly changing generalists, whereas metazoa are macroscopic, sexual, ecologically restricted, and morphologically

Abbreviations: SOP, sensory organ precursor; MT, microtubule; 3-D, three dimensional; CAM, cell adhesion molecule.

A commentary on this article begins on page 8417.

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diverse specialists with a history of repeated radiations (12). We may ask whether a capacity for rapid phenotypic change among metazoa can be reconciled with the conservation of most of the eukaryotic core cell biological mechanisms. We will argue that the conservation of these core processes for the past 530 million years is related less to the processes' own constraint, embedment, or optimization than to the deconstraint they provide for phenotypic variation of other processes, on the basis of which they are continually coselected. In this essay, we will identify the properties of conserved cellular and developmental processes that circumvent or reduce constraint. In particular, we will discuss versatile proteins, weak regulatory linkage, exploratory mechanisms, and genomic and spatial compartmentation, all of which confer flexibility and robustness on processes and consequently increase nonlethal phenotypic variation and evolvability.

Conservation and Principles of Cellular Evolution. The surprising conservation of core cell biological processes has permitted researchers to work on many different organisms in parallel. Students now learn that most processes are general for all eukaryotes and often for all life forms. Sequence conservation is so strong that over one-half of the coding sequences of yeast are recognizable in mice and humans (13). The actins of yeast and humans are 91% identical in sequence, for example, and the β-tubulins are 86% identical. Actin and tubulin are proteins engaged in numerous functional interactions at sites covering most of the protein surface. Their amino acid sequence changes are plausibly constrained.

When sequence is conserved, function is usually conserved, but conservation of function rather than sequence is the main issue because only function is selectable. Function is frequently conserved even when sequences differ substantially, as exemplified by divergent mammalian proteins which complement yeast mutants. Furthermore, even with an extensive change of amino acid sequence ($<$10% similarity), function and 3-D structure can be retained, e.g., among metazoan hemoglobins. Genotypic variation is often not matched by an equal functional variation. Reciprocally, small changes of sequence can result in large changes of function. Thus, although mutational change is needed for phenotypic change, the two are simply not related. Because only phenotypic change is subject to selection, we have to look beyond the quantity of sequence variation to understand how the structure and function of gene products have changed in evolution. Although the conservation of sequence and function has provided a molecular confirmation of Darwin's view that all organisms have descended from a common source, it has obscured the explanation for the profound differences of organisms. How can all this commonality serve as the platform for the immense diversification of metazoa?

Evolution of the Regulation of Cellular Processes. Although core cell biological processes are strongly conserved in eukaryotes, the control of these processes is not. In metazoan evolution, these processes have been brought under intercellular control regarding the time, place, and conditions of function. Regulatory processes have evolved greatly in metazoa. Regulation is imposed chiefly by inhibitions, and these inhibitions are often relieved by another inhibition, producing activation. In the cell cycle, e.g., conditional inhibitory proteins of the G_1 cyclin-dependent kinases tie the G_1-to-S progression to nutrient provision, growth factor availability, or lack of DNA damage (14). In metabolism, gluconeogenesis or glycolysis is activated through the inhibition of one of the opposing reactions of multiple futile cycles, depending on glucose availability. In gene expression, repression or activation can be imposed at any of several levels: nuclear localization of a transcription factor, dimerization of a transcription factor with itself or with a cofactor or with an inhibitory protein, competition of different transcription factors for sites on DNA, and reversible or long-lasting modification of chromatin. In regulated exocytosis, as occurs at the nerve terminal, a calcium-sensitive step is interposed in the normal pathway of unregulated secretion, making secretion contingent on the opening of calcium channels. In many of these cases, the evolution of regulatory inhibition of core processes seems relatively straightforward because many inhibitors are simply modified components of the process, lacking effector domains and acting as dominant negative agents.

Yet, eukaryotic cells also have several kinds of flexible and versatile systems of inhibition. The evolutionary emergence of these systems must have increased the ease with which inhibitions could be imposed on conserved processes and reduced the number of mutations needed for new regulatory connections. Calmodulin, an inhibitor used widely in eukaryotic cells, binds to many target proteins and transduces calcium signals. Although calmodulin itself is highly conserved in eukaryotes, its targets vary greatly in different kinds of cells. Calmodulin has the design features of a flexible versatile inhibitor (15). The sequences to which it binds in target proteins are fairly diverse (it is "sticky"), and it binds to these as a clamp with a variable expansion joint which can adopt different configurations when bound to different targets. Calmodulin undergoes a large change of 3-D conformation in response to calcium, resulting in altered contacts with the target protein. Its binding generally inhibits the target protein's function, and the subsequent binding of calcium to calmodulin usually lifts the inhibition. If the target (e.g., tropomyosin) is itself an inhibitor, then calmodulin-like proteins (e.g., troponin C) can act as activators. In other cases, the target protein may contain an internal inhibitory domain on the same polypeptide as its catalytic domain (e.g., CAM kinase II), and again calmodulin will appear to activate, by inhibiting the internal inhibitor (16).

The low sequence requirements for calmodulin's binding to targets (a result of its flexibility and stickiness) and its built-in capacity to alter target protein activity must reduce the number of random mutational steps needed for new targets to interact with calmodulin and generate new regulatory connections. Cadmodulin is effective without having to be highly specific. The property of versatility addresses one of the difficulties faced by Darwin in his theory of natural selection. He was concerned with "modes of transition." How was it possible for "organs of extreme perfection and complication" such as eyes or wings to develop by stepwise modification via intermediate forms offering little function for the organism (1)? He gave reasonable anatomical answers to this challenge, but the answers seem more compelling at the molecular level: highly flexible and versatile systems of inhibition can decrease the requirements for mutational change to produce new regulatory interactions. This is so even though the inhibitor itself is constrained in a particular way to function as a flexible versatile inhibitor.

Weak Linkage. Another property contributing to constraint reduction and hence to evolvability has been called "weak linkage" (8). Linkage refers to the coupling of processes, i.e., the dependence of one process on another. Strong linkage occurs when two or more proteins aggregate into an active complex or when one enzyme provides a product that is a substrate for another enzyme. Strong linkage often occurs in pathways involving successive chemical conversion or energy transfer or macromolecular assembly. Metabolism, for example, is based on the strong linkage of its many components. Steric requirements are high, and the complementary fit of surfaces of interacting components is precise. By weak linkage, we mean that the activity of a process depends minimally on other components or processes. Weak linkage is a characteristic of information transfer (regulatory) pathways, e.g., signal transduction, neural relays, or transcriptional control circuits, the very pathways elaborated in metazoan evolution. In these pathways, the components often have a switch-like capacity to exist alternatively in active or inactive states, and signals just

release the innate activity. Signals do not have to act instructively. One component of a signal transduction pathway may activate another that activates a third, but no material or energy of the first agent is found in the third. Such regulatory organization based on weak linkage facilitates a component's accommodation to novelty (to new activating or inhibiting signals) and reduces the cost of generating variation, as exemplified below for neural signaling and transcriptional control.

In neurons, the transmembrane potential controls voltage-sensitive calcium channels, regulating the cell's permeability to calcium ions. Calcium in turn regulates many processes, including secretion of neurotransmitters at the axon tip. Each cell is poised to secrete neurotransmitters when it is depolarized (as calcium enters) and poised not to secrete when it is polarized. These states are built into the nerve cell and are constrained. The cell is not waiting for materials and energy to become active, but only for a signal that opens any of many kinds of ion channels, triggering depolarization and secretion. The extended nervous system is based on weakly linked components. The membrane potential, on which all this weak linkage depends, is the algebraic sum of the behavior of (*i*) pumps, which act to return the cell to the homeostatic balance of ions; (*ii*) potassium-leak channels; and (*iii*) ion channels that are regulated internally and externally, including regulation by the membrane potential itself. The signaling channel does not have to interact directly with the calcium-dependent secretory machinery. It acts indirectly through depolarization. New channels that are added to the cell are immediately "understood" by the existing system because they all contribute to the same currency, the membrane potential. All that changes is the "when" and the "where" of transmitter secretion. The introduction of new channels, or new regulation of existing channels, does not threaten the viability of the cell or its poised capacity for neurotransmitter secretion.

Another major example of weak linkage is found in the regulation of eukaryotic transcription with its complex and highly conserved core components. The regulatory issue is the time, place, and conditions for a particular gene's transcription. In this case, a comparison with the related prokaryotic machinery is highly informative. In both cases, the stringent control of gene expression is ensured because the binding and activation of RNA polymerase at the transcription initiation site is contingent on the binding of other components. In prokaryotes, these other components must bind precisely (high sequence specificity) and close to the site of initiation (within 100 bases), and the number of regulatory components is small. However, the eukaryotic system admits to many transcriptional inputs from proteins bound at short enhancer sequences of different kinds, which can be at almost any distance within 50,000 bases from the initiation site and in either orientation. The *cis*-regulatory regions of metazoan genes are large and complex. Furthermore, many enhancer-binding proteins have limited affinity and rather low sequence specificity for these enhancer sites, and some bind with specificity only in the context of other proteins. Gene expression is controlled by many positively and negatively acting enhancer-binding proteins, whose own distribution may be spatially organized in embryos. Multiple inputs are very important in the expression of metazoan genes at different times, places, and conditions, that is, in response to multiple signals. These low requirements of enhancer sequence, location, and orientation, plus the receptiveness of the eukaryotic transcription machinery to various and multiple positive and negative inputs, endow eukaryotic, but not prokaryotic, transcriptional regulation with weak linkage. Weak linkage, which underlies the tolerance, flexibility, and robustnesss of transcriptional regulation, should have made it easy to add and subtract regulatory elements to eukaryotic genes and hence should have increased the evolvability of the system. Metazoa have indeed made heavy use of transciptional regulation.

Exploratory Mechanisms. Another class of deconstraining processes, which as a group we call "exploratory mechanisms," depends on epigenetic variation and selection (17). These too have properties by which their effective function has rather few requirements for other components and exact conditions. They generate a large number of configurations or states from which other components select the single functional outcome.

The best known of the exploratory systems is vertebrate adaptive immunity, which can produce any one of 1 million different antibodies with high affinity for any of an even larger number of antigens, without the animal's foreknowledge of the universe of antigens. In this system, variation in T cell receptors is achieved by random recombination and sloppy joining among several genes. Each T cell expresses a single recombinant T cell receptor that is activated by an antigen-presenting cell with the appropriate antigen, and therefore, the cell is stimulated to proliferate. Here, variation (by recombination) comes first, followed by selection (reaction with the antigenic peptide presented on another cell). Similar considerations hold for the generation of diverse IgGs of B cells.

An exploratory system in cell biology, based on similar principles, is the morphogenesis of the microtubule (MT) cytoskeleton, which plays multiple roles in the cell. In one of these, spindle MTs connect to the kinetochores of numerous chromosomes and mediate chromosome segregation to the spindle poles. Specific connections must be made despite the facts that chromosomes can be located anywhere in the cell at the onset of mitosis and that the cell can have any of a wide range of sizes and shapes. MT arrays are generated by polymerization of MTs in random directions from the centrosome. Each MT, as it polymerizes, is only transiently stable unless it encounters a stabilizing activity that binds to its growing end. Otherwise it depolymerizes back to the centrosome, and a new MT is initiated in a random direction. When a MT tip fortuitously hits a kinetochore, it is stabilized by proteins there; hence, the chromosome inevitably becomes attached to a MT despite its initial random location in the cell (18). Variation is present in the random spatial orientation of the polymerizing MTs. Selection occurs in the stabilization of a particular MT of a particular orientation. Such a process is robust because it achieves a functional state whatever the initial arrangement and number of chromosomes or the morphology of the cell. It is constraint-reducing because its requirements are low for achieving a specific functional state. The capacity to polymerize, depolymerize, and be stabilized is built into the highly conserved and constrained tubulin protein. Yet the uses of MTs are highly diversified. Because the process is so robust, it need not be modified when the cell's morphology is modified by other mutations. Hence, the process facilitates the evolutionary change of other components by deconstraining changes, itself having an unlimited range of possible configurations and a broad receptivity to stabilizing conditions. The generation of new cell morphologies simply requires novel placement of stabilizing activities rather than the remaking of the process of MT nucleation and assembly. Thus, cell morphogenesis, which would seem to require many fundamental changes to bring together specifically placed and interacting components, entails the same core mechanism in which flexibility and robustness are conserved.

The immune system and MTs are extreme examples of epigenetic variation and selection. Variation is completely random and unlimited (in the space of antigen variety or the cell's volume, respectively), and selection occurs only after the variation process and independent of it. Nothing biases the recombination system toward a given T cell receptor or antibody structure, and nothing guides MTs to chromosomes. Other exploratory mechanisms in embryonic development play an important role in deconstraining evolutionary change.

For example, in the genesis of neuronal connections, axonal growth cones are exploratory as they engage in pathfinding toward a target site; they extend and retract numerous microspikes, some of which make contracts with stabilizing components of the substratum. Initially, the axonal connections to the target site are excessive and approximate. The final sorting out and pruning depends on neuronal activity, a functional selection from exploratory connections. Although the gross structure of the CNS is specified, many local features achieve organization by variation and selection.

In embryonic development, migrating and proliferating cell populations have been very important for evolutionary change. Two major examples can be cited for vertebrates: the limb (or fin) and the neural crest cell population. Functional selection is important in the vertebrate limb, a structure that has undergone rapid morphological evolution, ranging from wings to flippers. The limb is a complex structure with precisely placed bone, cartilage, muscle, nerves, and vascular elements, and one might think it is difficult for such a structure to change in evolution. The basic structure of the limb depends on serial cartilaginous condensations formed under the direction of Hox genes and other selector genes. Once the condensations are generated, the organization of the musculature, nerves, and vascular system arises through exploratory processes involving precursor cells randomly migrating into the limb rudiment. Muscle cells arrive from nearby somites and adapt to the condensations. Their proliferation is presumably controlled by locally produced factors. Axons then extend in from spinal ganglia, and stabilization occurs through synapse formation with target muscles and exposure to locally secreted growth (anti-suicide) factors. Then the vascular system invades the limb. Angiogenesis is a well established example of functional selection. Although large vessels are probably placed by instructive processes, moderate and small vessels are not, as indicated by bilateral differences in pattern. In the vascular system (and in the analogous tracheal system in insects), local oxygen deprivation may play a role in controlling the growth and branching of moderate and small vessels (19, 20). The exploratory nature of these systems is very deconstraining. Evolutionary modification of vertebrate limb shape and size is reduced mostly to the mutational modification of the cartilaginous condensations. It need not be simultaneously accompanied by mutationally derived changes in the muscle, nerve, and vascular systems, which can accommodate to any of a wide range of limb sizes and shapes.

Major innovations in vertebrate anatomy also have been achieved by neural crest cells, which are responsible for much of the development of the face, skull, peripheral nervous system, and pigmentation. Neural crest cells are exploratory cells in that they can: (*i*) migrate throughout the embryo at an early stage and settle at any of many sites, (*ii*) receive a wide variety of local signals at the sites, (*iii*) proliferate extensively in response to signals, and (*iv*) cytodifferentiate to a wide variety of cell types in response to signals. Although these cells gain an initial broad anterior–posterior identity from the products of Hox genes expressed in their regions of origin bordering the neural plate, their final proliferation and cytodifferentiation are selected from their large repertoire by local signals at the particular dorsal–ventral site where they chance to settle. As sites in the periphery change in evolution, neural crest cells are always available to find them and respond to their local signals. The first uses of neural crest may have been for dentine armor in the early jawless fish, the ostracoderms, but later these cells were used for structures such as the teeth (dentine), gill arches, and jaw. The huge head shield of the dinosaur *Triceratops* and the 3-m antlers of the extinct Irish elk are almost certainly neural crest derivatives. Clearly, the early selection for the neural crest did not anticipate its capacity for forming antlers and jaws. It must have been a high evolvability of these cells, based on their capacity to migrate, proliferate, and respond in any of a variety of ways to signals from other cells of the embryo, that allowed them to play such an important role in vertebrate evolution.

To conclude this section, exploratory mechanisms deconstrain evolutionary change by virtue of their low requirements for achieving complex functional outcomes. Their properties of flexibility, robustness, and weak linkage are highly conserved. They are able to tolerate change because they are physiologically robust. This robustness also should mitigate potentially damaging mutations, thus improving the fitness of the organism. By generating many states, any of which may be selectively stabilized in different ways, they also reduce the number of mutational changes a system has to undergo to achieve new functional interactions and morphologies. Hence, they would be expected to contribute to evolvability.

Compartmentation, Redundancy, Robustness, and Flexibility. Although multicellularity has been attempted many times in both prokaryotes and eukaryotes, the metazoa have been unusually successful in generating complex cell differentiation and tissue organization. Members of this monophyletic group share many characteristics, including a collagenous extracellular matrix, and true epithelia with tight junctions. Early metazoa, through natural selection, achieved extensive control over the milieu of internal cells and generated many physiologically selective micro-environments in that milieu. Multicellularity was one of the great steps in evolution in so far as it led to the specialized use of cells at different places in the population. Spatial differentiation, organized through cell–cell signaling, presumably evolved concurrently with cell types because random arrangements of different specialized cells would have been of little advantage.

Compartmentation is an important and widely recognized stratagem of evolvability in organisms with a complex spatial organization of multiple cell types (4, 6, 21, 22). Like weak linkage and exploratory mechanisms, compartmentation reduces the interdependence of processes, consequently reducing the chance of pleiotropic damage by mutation and increasing phenotypic variation. Here, we define genomic compartmentation as the division of the cell's total genomic potential into partially independent subsets of expressed genes, as seen in cell differentiation. The use of the cell's conserved core reactions, encoded by these genes, is compartmentalized by the expression of genes in different combinations and levels. In metazoa there are at least four types of cell differentiation: cytodifferentiation, spatial differentiation, temporal differentiation, and sexual dimorphism at the single cell level. The second is unique to multicellular organisms, whereas the first and third are greatly exaggerated in them, compared with single-celled eukaryotes. In each of these types, various transcription factors and enhancer binding proteins interact with complex *cis*-regulatory regions of genes (often longer than the coding sequences themselves) and determine which genes are expressed (and in the case of differential splicing, what parts of genes are expressed). This compartmentation of expression is contingent on external signaling factors, which in metazoa are provided by other cells, and on internal factors (active transcription factors) brought forward in the cell lineage from previous stages of development. As discussed in the section on transcriptional control and weak linkage, factors binding at different subregions of a *cis*-regulatory region can act independently, and so a single gene can be expressed at many different places, times, and levels, depending on different conditions. The different conditional responses of a single gene's expression can be selected independently. Gene duplication is also a means toward genomic compartmentation. Initially the duplicated genes would be fully redundant, but with time, one or the other member can diverge in its conditions of expression (mutations in the *cis*-regulatory region) or in the specialized function of its protein (mutations in the coding region), passing through various degrees of partial

redundancy of function. Redundancy protects old functions as new ones arise, hence reducing the lethality of mutation (23).

Cytodifferentiation has been well studied in the muscle cell, which has exaggerated and specialized the common actomyosin contractile machinery of all eukaryotic cells. Specification of cells of the vertebrate muscle lineage, first as myoblasts, requires the expression of two myogenic master switch genes (*MyoD* and *Myf5*), whose encoded transcription factors in turn activate the genes of two other muscle-specific transcription factors (MRF-4 and Myogenin). These eventually activate muscle-specific genes (actin, myosin), triggering overt cytodifferentiation into myocytes (24). These four myogenic genes are of related sequence, probably evolutionarily diverged from a single sequence, and their encoded products are part of a partially redundant and complex crossactivation network in which the accumulation of any one active protein to a threshold level leads to the autoactivation of the entire network, and hence to a self-maintaining myoblast. Many cells of the embryo initially express one or more of these genes at a low level, but only a few cells sustain this expression through subsequent steps to achieve the autoactivated state of a stable myoblast. There are many ways for embryonic cells to lose specification. Many signals including local proliferative factors down-regulate this low level of expression, whereas local muscle inducing factors are required to up-regulate it. Many paths lead to muscle differentiation, and even within the same organism, different myogenic genes are expressed first in different muscle forming regions (25). By maintaining a large pool of prospective muscle cells (an equivalence group, a kind of cellular redundancy) and using several cycles of positive and negative regulation of a control circuitry with partially redundant crossactivating components, the developmental system can respond in a robust way to different local conditions, damage, spatial misallocation of cells, or mutation.

There are many exquisite spatial arrangements of cell types in metazoa, all with selected functions. In the development of such patterns, a long period of specification of arrangements precedes the actual cytodifferentiation and involves successive steps (refinements of pattern) of intercellular signals and intracellular responses. An enduring question about such developmental mechanisms has been, "How can they remain stable within each generation and yet be evolutionarily modifiable?" Research of the past decade has shown that pattern formation processes are developmentally robust but in principle easy to modify in evolution. Perhaps no pattern of a single cell type is as complex as that of the 5,000 precisely arranged bristles on the million-celled surface of the adult *Drosophila* (26). Each region of the surface has its unique local array of bristles. Although bristle patterns are very reproducible within a species, they differ between males and females and are very diverse among closely related species. A differentiated bristle arises from a sensory organ precursor (SOP) cell. An SOP is established as a committed cell when members of a network of proneural genes (*achaete*, *scute*, *lethal-of scute*, and atonal) become autoactivating in their expression. (The genes are called proneural because the bristle complex contains a nerve). Like the myogenic master control genes, these are partially redundant genes of related sequence, standing atop a hierarchy leading eventually to a specific cytodifferentiation, in this case a bristle. Until autoactivation is achieved, the genes depend on many other signals, external and internal, for continuing activation. The proneural proteins, which are all transcription factors, are opposed by inhibitory proteins, which resemble the proneural proteins but lack DNA binding domains. These proteins, inhibit SOP formation when their genes are expressed at levels exceeding those of the proneural genes. Each of the pro- and anti-neural genes has an extensive *cis*-regulatory region at which regulatory inputs are received from local intercellular signals (Wg, Hh, and Dpp), intracellular Hox gene products, or sex-specific gene products. The multiple positive and negative inputs divide the embryo into thousands of nonequivalent regions, each of 10–100 cells, and each region does or does not maintain a cluster of prospective SOPs. Even at this point, not all SOPs of a cluster will make it through to bristle differentiation, for within these regions, a subsequent lateral inhibition step occurs involving the Notch/Delta-signaling system, by which SOP formation can yet be suppressed. One or a few well spaced SOPs, which have finally achieved autoactivation of the proneural genes, may then remain in the region for cytodifferentiation during metamorphosis of the larva to the fly.

This conserved patterning process displays versatility, physiologic robustness, and high evolvability. It can receive and discriminate myriad inputs. A twofold reduction in cell number (produced by starvation) causes surprisingly minor perturbations of pattern (27). A local lowering of the anti-neural gene products or a raising of proneural products will cause an expansion of the bristle pattern, and the opposite changes will contract the pattern. Lateral inhibition will keep the bristle spacing normal. Combinatorial changes in secreted signals or in domains of Hox or other selector gene expression may change a pattern in one region, but other regions will be unchanged. The specification of the location of the SOP occurs well before the cytodifferentiation of the bristle (the two processes are temporally compartmentalized), so that a modification of the position of the SOP by mutation or developmental imprecision will never compromise the function of the bristle. This system has the capacity to distinguish hundreds or thousands of different locales in the insect surface epithelium, and give a yes/no response to each. The flexibility and robustness of this conserved system would seem related to its selected use for many different pattern outputs in response to many different local circumstances within the same individual. A system with such great flexibility of use in one individual would seem exquisitely suited to generate, by modest mutation, different patterns in different individuals in evolution.

Although genomic compartmentation and redundancy may have been selected for the physiologic robustness they confer to the development and physiology of complex metazoa, they also facilitate evolutionary change by making various cell populations independent, reducing the chance of lethal mutation and increasing the independence of variation and selection within a compartment. Because of compartmentation, changes in extracellular or intracellular signals are more likely to result in local elaboration of new morphologies than in a catastrophic failure of global organization.

Developmental Compartmentation of the Conserved Phylotypic Stage. Evolvability is seen also in the grand strategies of organismal development, particularly in the conserved use of a compartmental organization of the body plan. Within each of the 30–35 phyla, all members share a characteristic body plan that is first evident in development at an intermediate stage called the phylotypic stage. Although it has been suggested that the common body plan within a phylum may just be an artifact of "random phylogeny," in which taxonomists endow the residue of common characteristics with exaggerated significance (28), modern molecular studies have given the conserved body plan and the phylotypic stage much more meaning. In arthropods for example, the conserved stage, called the segmented germ band, is much more than a morphological composite of distinguishable parts (21, 29). It is a spatially arranged collection of 50–60 self-sustaining compartments of developmental processes of great versatility. Each compartment has an identifying set of expressed selector genes for transcription factors (such as the *ems*, *otd*, and Hox genes) and for secreted signals. The compartments include multiple segmental domains, each with an anterior and posterior portion, the nonsegmental terminal domains (acron and telson), and several dorsal–ventral subregions including the three germ layers. Each is largely independent from other compartments

in the subsequent development occurring within it, and in its evolution.

This body plan is easily observed in long germ band insects in which it is generated nearly synchronously in the embryo. In short germ band insects where thoracic and abdominal segments arise later and successively from a proliferating zone of cells, there is a temporal lag from the anterior to posterior ends. Yet the basic pattern is very similar in all arthropods (30). Chordates also have a phylotypic body plan and stage, the pharyngula, with a segmented mesoderm (the somites), an anterior/posterior series of regions distinguished by *emx*, *otx*, and Hox selector gene expression, and a dorsal–ventral organization strikingly similar to arthropods (11, 31). The inverse orientation of this dimension in chordates compared with arthropods reflects merely the organism's preferred orientation with respect to gravity, not a basic difference of this aspect of the body plans. Beyond these similarities, the pharyngula differs of course from the segmented germ band in terms of gill slits, a post-anal tail, a notochord, and a dorsal hollow nerve chord.

Because representatives of virtually all 30–35 modern phyla were present 530 million years ago in the mid-Cambrian period, body plans and phylotypic stages must have been fixed by that time. For a period 10–100 million years before then, a great radiation of large, forcefully moving metazoa took place into new and unoccupied niches and then stopped. Since then, there have been extensive diversifications of the larval and adult stages of members of each phylum as seen in the great diversity of classes and orders of modern phyla. These diversifications occur at developmental stages after the phylotypic stage has formed, and they are built upon the body plan. They were absent in the pre-Cambrian founders of the phylum. Also, there have been extensive diversifications of the egg and early stages of development before the phylotypic stage is formed. Thus, evolution since the mid-Cambrian has involved modifications before and after the phylotypic stage but not of the stage itself.

Several explanations have been given for the transient burst of diversification of body plans, including that the pre-Cambrian was a special period for mutation, and when this period ended, mutagenesis declined. However, quantitative analysis of the morphological characteristics of the Cambrian arthropods, showed no unusual degree of disparity, suggesting that the tempo of evolutionary change at that time was not unusually great (32). Rather the emergence of the different body plans seems related to an ecological breakthrough into rich, diverse, empty niches by large metazoa (for that time) descended from a predecessor with great evolvability of compartmental body organization. The halt to body plan diversification by the mid-Cambrian may reflect the saturation of niches and increased competition for resources by phylum members with recently acquired appendages, mouthparts, and improved sense organs, the various first additions to the body plan. As for the conservation of these body plans from 530 million years to the present, some authors have invoked constraint, namely that: (*i*) the body plan at the phylotypic stage is so embedded in the organism's development that any modification is lethal, and (*ii*) development just before this stage involves highly interdependent, noncompartmentalized processes, susceptible to mutational damage (4). We believe, however, that this is only a partial explanation, and that the body plans have survived because they have continued to provide a function that has been under continuous selection, namely, the many diversifications afforded by the body plan's evolvability.

To appreciate why body plans and phylotypic stages may be conserved, one needs to understand not only their use in later development but also their unusual formation in early development. We will consider their formation and then their function. To take just the *Drosophila* egg, simple preconditions of that egg involving the localization of four gene products (two RNAs and two proteins) in five different places are sufficient to set up the dorsal–ventral axis, the anterior–posterior axis, and the anterior and posterior termini of the embryo. Early developmental processes bootstrap on the minimal spatial organization of the egg to generate spatially complex intermediate reactions, which in turn activate the compartment-specific genes of the body plan, namely the Hox genes, segment polarity genes, terminus genes, and those of the dorsal–ventral compartments. The early pattern starting from the four localized gene products of the egg elaborates into the 50 or 60 different spatial compartments of the body plan. Once compartment-specific genes are activated by these transcription factors, they become autoactivating, much like the myogenic genes of the myoblast or the proneural genes of the SOP. The complex, poised circuitry of the compartmental networks is only weakly linked to the prior reactions, with the consequences that (*i*) early activating inputs are no longer required once a compartment is autoactivating, (*ii*) the compartment circuitry is readily activated by any of a number of inputs to various places in the circuitry, and (*iii*) the spatial organization of the early inputs is simpler than that of the compartments themselves. In general, the compartments set very few requirements on early development.

When comparing *Drosophila* to other arthropods, there are marked differences in the development leading up to the common phylotypic stage. The phylotypic body plan seems special in its approachability from many different directions, and this property reflect the weak linkage and minimal requirements that compartments set on prior development. For example, short germ band insects dispense with several of the early steps of *Drosophila* axis specification. Furthermore, in parasitic insects such as polyembryonic wasps, whose early single embryo fragments into 2,000 morulae that develop to a clone of 2,000 larvae, the early stages lack the chorion which in *Drosophila* contains the spatial information for the dorsal–ventral dimension and the termini; yet, the wasp egg still forms a segmented germ band embryo (33). Also among vertebrate orders, the early steps of axis specification and morphogenesis are very divergent but lead to the common pharyngula. In placental mammals, there is no known asymmetry of the egg that is used in later patterning, whereas in most amphibians, many fish, and cyclostomes, the initial animal–vegetal asymmetry of the egg is modified by a self-organizing cortical rotation process to generate provisional axes. Also, there are many differences in gastrulation. Nonetheless, a similar phylotypic stage is set up.

In light of the variety of early development, we suggest that the network of reactions that characterizes the body plan of the phylotypic stage makes few demands on the reactions at earlier developmental stages for its initiation. The early embryo must generate some initial polarities, but they can be simple, *ad hoc*, and diverse. Once orientated, placed, scaled, and activated by these reactions, the compartments of the phylotypic stage become self-perpetuating. The weak linkage, physiological robustness, and nonoverlapping use of components (a temporal compartmentalization) of the early developmental processes permit major modifications of the egg in evolution while still meeting the developmental demands of the phylotypic body plan. Because egg evolution is closely tied to reproductive specializations in the life history of the organism, it is under strong selective pressure. Hence, we see radically different eggs of closely related organisms developing to the same phylotypic stage, comprised of the same network of spatially compartmentalized reactions. We suggest that a conserved property of the body plan, attributable to its selected constrained circuitry, is its ease of formation.

As a further important property, the phylotypic body plans of arthropods and vertebrates, as well as presumably of the other 30-some phyla, also serve as the platform for the wide

diversity of later development that distinguishes the classes and orders of a phylum. The success of lineages of organisms has depended greatly on the elaborations occurring in development after the phylotypic stage. They are the most prominent feature of adults. (No extant organism gets by solely with its unadorned body plan, as sufficed in the pre-Cambrian.) Indeed, in arthropods, the phylotypic stage has allowed the development and evolution of the head, appendages, genitalia, and the differences of the larval and adult stages. In chordates the pharyngula has allowed the evolution of the limbs, head, jaws, and peripheral nervous systems. As discussed above, the neural crest is a migratory, responsive, and multipotent cell population that explores the compartments of the pharyngula, and upon settling at various sites, makes many additions to the body plan. The development of each add-on structure is semi-autonomous once it is activated (i.e., they too are compartmentalized), but it initially depends on signals from the compartments of the phylotypic stage for placement, orientation, scale, and timing, that is, for overall organization. Because their developmental function is based on selector gene products and secreted protein signals, compartments are very versatile. Any kind of gene expression and subsequent development is possible within each, and this would apply independently to all compartments of the body plan.

Although constraint may be part of the explanation for conservation of the phylotypic body plan, it is a particular kind of selected constraint that generates versatility in the use of compartments and deconstraint in their formation. From this perspective, a phylotypic body plan is a conserved set of spatially arrayed reactions, constantly being selected for the diversity of life histories it supports, including early stages of embryonic nutrition and protection as well as adult forms.

Conservation and Deconstraint. Constraint, as noted, arises from the molecular interactions underlying a component's function. The more exacting, instructive, and numerous the requirements for function, the more constrained the component is to a change of amino acid and/or base sequence. New functions bring new constraints. With more functions and interactions, the susceptibility of a process or component to damage from random mutation increases and the possibilities for viable phenotypic variation decrease. If constraint is inevitable, we must ask why particular constrained mechanisms have been conserved and others have not? We argue that these processes are conserved because they deconstrain phenotypic variation in other processes, and hence facilitate evolutionary change.

We have considered various conserved processes of metazoa that are repeatedly used for different purposes and at different times, places, and conditions in the same organism. These mechanisms are flexible, robust, and versatile. Whatever pre-Cambrian conditions selected these wonderful processes also selected the deconstraining properties that allowed the further use of these processes in individuals of a given clade of organisms and also with modest modification in different clades. In addition to facilitating the use of processes in different contexts, many of these properties add to physiological robustness by minimizing the dependence of a process or component on intra- and intercellular conditions, hence minimizing lethal damage caused by mutation and reducing the number of mutational changes needed for phenotypic novelty. The following are summaries of the various means of deconstraint:

1) *Flexible versatile proteins* such as calmodulin can interact with a variety of targets and can readily impose inhibitions and activations. Similar broad specificity for targets is seen for many conserved protein kinases as well, the key agents connecting signaling pathways to targets in the eukaryotic cell. For all of these, it appears that only a small amount of mutational modification is needed for a potential target protein to become susceptible to regulation.

2) *Weak linkage* occurs widely in the information relay pathways of signal transduction, transcriptional control, the nervous system, and development. In many cases, conserved components are constructed with switch-like, two-state properties. These properties entail internal constraint of the poised components, but they are externally deconstraining because agents interacting with the components have only to select or trigger the activity already latent within the component. Instruction is not required. Requirements are low for the establishment of new regulatory connections, and multiple and indirect inputs are the rule. In some weakly linked systems, redundant components are used, and damage to critical components is minimized. As a result, the experimental knockouts of important genes, such as individual Hox genes in vertebrates or the MyoD gene of muscle specification, have unexpectedly mild phenotypes. These robust, flexible systems increase the capacity for the organism to accumulate nonlethal genotypic and phenotypic variation and minimize the changes needed for new regulatory connections.

3) *Exploratory systems* like angiogenesis, nerve outgrowth, neural crest cells, and MT-based morphogenesis (and even behaviors such as ant foraging) are based on epigenetic variation and selection. The conserved variation process generated an enormous variety of configurations or states, and has a broad receptiveness to selective stabilizing conditions. Requirements for effective function are minimal, and the process tolerates altered conditions caused by environmental insult, the use at different times and places within the individual organism, and mutations. Although the variation machinery of the process is conserved, the selective stabilizing agents can vary widely and readily, without the need for a parallel change in the variation machinery, when new morphologies and interactions are established in evolution.

4) *Compartmentation* includes genomic compartmentation (various combinations of expressed genes), the spatial compartments of the body plan, and equivalence groups found at many stages of development. Compartments buffer against developmental inaccuracy and reduce pleiotropic damage from mutation. Such systems facilitate evolutionary change by preserving viability when the size, anatomy, or placement of cells of the embryo changes by environmental, developmental, or mutational means and by allowing independent variation and selection in units smaller than the whole of the organism.

Thus, as a general principle, deconstraint is conferred by these conserved mechanisms. They seem designed to minimize the interdependence of processes and to spread the potential for phenotypic variation unevenly among all of the organism's activities. Some processes have been highly conserved, not so much because they cannot change, but because they are flexible and robust mechanisms that support change and variability in other processes. Evolvability, we suggest, is the legacy of the deconstraints afforded by conserved processes. Nonlethal genetic and phenotypic variation may accumulate in the vicinity of conserved processes with these properties.

Can There Be Selection for Evolvability? The proposal that evolvability has been selected in metazoan evolution raises difficulties because it seems to be a trait of lineages or clades rather than individuals. Clade selection is often considered an "explanation of last resort." Also, evolvability seems to confer future rather than present benefit to the individual. If these difficulties can be surmounted, the view of cell biological and developmental processes in terms of evolvability offers an opportunity to understand better not only the phenotypic variation component of evolutionary change but also the deeper selected function of these processes. Although difficult to prove, we believe that selection for evolvability has had three components, one related to individuals, a second related to individuals and clades, and a third related to clades, as follows.

(*i*) For the individual, various flexible, robust processes may have been selected because they contributed directly to phys-

iological fitness and complex development. Such processes tolerate variability of cell number, cell position, the conditions set by other processes, and the environment. Such processes also would be selected in complex organisms evolving by descent with modification of preexisting processes (the alternative being *de novo* modification). As a nonselected byproduct, flexible robust processes would facilitate phenotypic variation and hence evolvability. Individuals with such processes may have generated phenotypic novelty with fewer mutational changes than those with nonrobust, nonflexible processes.

(*ii*) Individuals with such processes would be buffered from the lethal effects of mutation, and the population would carry more nonlethal genetic variation. Genetic variety would be available to individuals directly and through mating with other clade members (for gene combinations). Clade members with such conserved processes might be selectively favored because they could diversify at a greater rate under selective conditions, i.e., could display greater evolvability.

(*iii*) Greater evolvability caused by such processes would have provided a clade level advantage for survival when rapid radiations occurred, not only when niches were emptied by massive extinction but also when new ecological domains were entered, such as air for winged insects or birds and wetlands for the first amphibia. Organisms may frequently undergo local small extinctions and population outgrowths, and ecological upheavals have led to several widespread extinctions of large groups of organisms. Lineages with such histories could especially benefit from the ability of clades to radiate into new or emptied environments, i.e., from evolvabilty. Chordates and arthropods have such a history. Perhaps evolvable mechanisms were further selected in clades exposed to frequent radiations. Mechanisms of evolvability, even if they had other costs, might allow a preemptive exploitation of the environment and therefore confer further evolutionary advantage.

From another perspective, as we look at breakthroughs in metazoan design since the pre-Cambrian, they seem to involve a succession of new attributes of evolvability, as if evolvability has itself evolved. When multicellularity was new among the early metazoa, the basic aspects of epithelia, the extracellular matrix, and control of the intercellular milieu were varied, selected, and conserved. Then a variety of intercellular signaling pathways and transcriptional regulatory circuits arose by variation and selection and were conserved. Then cell types, body organization, and various aspects of development arose. By the mid-Cambrian, the phylotypic body plans arose and were conserved. Thereafter came the additions to the body plans, such as appendages, neural crest derivatives (vertebrates), and numerous reproductive specializations of the early stages of development. In each round of variation, selection, and fixation, it appears that during the fixation period there was a selection for properties of robustness and versatility of the new-found processes. These properties then facilitated the generation of phenotypic variation used in the next round, and we think, ensured the conservation of the flexible robust process itself, which was further selected with those new variations. Organisms with processes lacking robustness and versatility presumably lost out in the next round because of their inability to retain as much genetic variation and to generate selectable phenotypic novelty with as few mutational changes. Added to this is the preemptive argument that a clade of organisms still perfecting its body plan at a time when others (with perhaps less perfect plans) had already started originating appendages and mouthparts was now at a selective disadvantage, compared with a previous time. Evolving organisms changed the selective conditions in a direction reflected by the successive times that various processes were first conserved.

Today, we see the survivors of lineages that underwent multiple radiations. These lineages have diversified by maintaining a core of highly conserved processes and modifying others. The core processes have unusual capacities to deconstrain change in other processes and components. This has proven to be a powerful strategy for the variation side of Darwin's variation and selection principle of evolution.

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