The theory of facilitated variation

John Gerhart*† and Marc Kirschner‡

*Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720; and ‡Department of Systems Biology, Harvard Medical School, Boston, MA 02115

This theory concerns the means by which animals generate phenotypic variation from genetic change. Most anatomical and physiological traits that have evolved since the Cambrian are, we propose, the result of regulatory changes in the usage of various members of a large set of conserved core components that function in development and physiology. Genetic change of the DNA sequences for regulatory elements of DNA, RNAs, and proteins leads to heritable regulatory change, which specifies new combinations of core components, operating in new amounts and states at new times and places in the animal. These new configurations of components comprise new traits. The number and kinds of regulatory changes needed for viable phenotypic variation are determined by the properties of the developmental and physiological processes in which core components serve, in particular by the processes' modularity, robustness, adaptability, capacity to engage in weak regulatory linkage, and exploratory behavior. These properties reduce the number of regulatory changes needed to generate viable selectable phenotypic variation, increase the variety of regulatory targets, reduce the lethality of genetic change, and increase the amount of genetic variation retained by a population. By such reductions and increases, the conserved core processes facilitate the generation of phenotypic variation, which selection thereafter converts to evolutionary and genetic change in the population. Thus, we call it a theory of facilitated phenotypic variation.

conserved genes | phenotypic variation | physiological adaptability | regulatory change

We will discuss the means by which animals have generated developmental and physiological variation since Cambrian times. In the course of their descent from a common ancestor, animals have diverged in their anatomy and physiology by the gradual accumulation of selected heritable modifications, their phenotypic variations. Although such variation is indispensable to evolution, Darwin conceded that "our ignorance of the laws of variation is profound" (1), and 150 years later the mode of its generation remains largely unknown. Phenotypic variation is thought to affect all aspects of an animal's phenotype and to be "copious in amount, small in extent, and undirected" with regard to selective conditions (2). Most of these characterizations go back to Darwin himself. As Gould has noted (2), they accord well with selection's primacy as the creative force in evolution, refining chaotic, profligate variation into exquisite adaptations. However, they afford little insight into the generation of phenotypic variation, and they raise questions about how copious, small, and undirected variation really is. Although small in extent, heritable phenotypic variations need be significant enough to be selected, and, if complex change entails numerous sequential phenotypic variations, evolution may be impeded. An example we will pursue later is that of the species of Darwin's finches that diverged in the Galapagos from a common ancestor. The beaks of some species are large and nutcracker-like, and those of others are small and forceps-like. As Darwin did, we too might imagine that many small heritable beak variations accrued slowly in the different species to create large observable differences. Small variations are arguably the only viable and selectable ones, because they would allow the upper and lower beaks, the adjacent skull bones, and head muscles to coevolve with each other in small selected steps, thereby maintaining viable intermediate beaks along the paths to the nutcracker and forceps forms. Repeated selections would be needed to coordinate the numerous, small, independent beak and head changes, all requiring genetic change. Is this an accurate appraisal of the paths of change? Or might the finch's own means of beak development coordinate many changes, allowing larger viable variations and a simpler, more rapid beak evolution? Insight into the mode of generation of variation could answer such questions about the size, abundance, and directedness of phenotypic variations.

Research of the modern era has revealed that heritable phenotypic variation requires genetic change, that is, DNA sequence change. Changes occur throughout the genome, although perhaps not at uniform frequency, and include changes of single bases or short sequences or even long segments of DNA (3). Some genetic changes are lethal, some are neutral, and fewer are viable and selectable. Furthermore, the understanding of variation has advanced with the knowledge that DNA sequences encode RNA and protein, because the latter two would bear the marks of DNA sequence change and, in principle, alter the phenotype. Also, discoveries of gene regulation have opened the possibility of important evolutionary changes in nontranscribed DNA sequences, as well. Still, there are no "laws of variation" regarding its generation, only a black box of chaotic accidents entered by genetic variation and occasionally exited by selectable phenotypic variation.

In the past 20 years, enormous insights have been gained about the development and physiology of animals, namely, about the generation of their phenotype from their genotype, the kind of information eventually needed to explain and predict phenotypic change from genetic change. From these advances, can something now be said about the nature of phenotypic variation and its dependence on genetic change? What is really modified in descent with modification? Have all components of a new trait been modified a little, or a few elements a lot while others not at all? Are many genetic changes needed for a modification of phenotype or only a few? Are there preferred targets for change? Are there cryptic sources of variation? These questions require concrete answers that can come only from in-depth studies of the phenotype, that is, the animal's development and physiology.

We propose that the phenotype of the organism plays a large role in (*i*) providing functional components for phenotypic variation and (*ii*) facilitating the generation of phenotypic variation from genetic change. We outline a set of concepts from others and ourselves, organized in a theory of facilitated variation, to connect genetic and phenotypic variation (see ref. 4 for a longer presentation). Like other theories (5–7), it identifies regulatory changes as ones particularly important for animal evolution, but unlike others it also emphasizes the targets of regulation.

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†To whom correspondence should be addressed. E-mail: jgerhart@berkeley.edu.

Table 1. The metazoan toolkit of conserved functional components and processes: When did they first arise in evolution?

First arose in evolution	Conserved functional components and processes
Three billion years ago, in early prokaryotic organisms	Components of energy metabolism, biosynthesis of the 60 building blocks, DNA replication, DNA transcription to RNA, translation of RNA to protein, lipid membrane synthesis, transmembrane transport
Two billion years ago, in early eukaryotic cells	Components of the formation of microfilament and microtubule cytoskeletons, motor proteins moving materials along the cytoskeletons, contractility processes, movement of the cell by cilia and ruffling membrane action, shuttling of materials between intracellular organelles, phagocytosis, secretion, chromosome dynamics, a complex cell cycle driven by protein kinases and protein degradation, sexual reproduction with meiosis and cell fusion
One billion years ago, in early multicellular animal life forms	Components of 15–20 cell–cell signaling pathways, cell adhesion processes, apical basal polarization of cells, junction formation, epithelium formation, specialization of cells toward physiological ends, some developmental processes of the single-celled egg to the multicellular adult
Near pre-Cambrian, in animals with early body axes	Components of complex developmental patterning, such as anteroposterior axis formation (Wnt/Wnt antagonist gradients) and dorsoventral axis formation (Bmp/antagonist gradients), inductions, complex cell competence, additional specialized cell types, formation of the body plan's map of selector gene compartments (both transcription factors and signaling proteins), various regulatory processes

We include four steps from genetic variation to viable phenotypic variation of anatomy and physiology, and we wish to show at which steps the facilitation of variation occurs, and how it occurs. First, as widely accepted, genetic variation arises from recent mutations and rearrangements of the genome and from standing genetic differences arranged in new combinations by sexual reproduction. Second, particular genetic variations then lead to regulatory changes, namely (*i*) changes of DNA sequences at cis-regulatory sites; (*ii*) changes of DNA at sites transcribed into RNA regulatory regions, such as those for RNA stability, translatability, and splicing (including microRNA processing); or (*iii*) DNA sequences transcribed and translated into protein regulatory regions, such as those for posttranslational modification, protein activation or inactivation, stability and degradation, or for binding regulatory agents and transducing their effects. Third, these regulatory changes impact "what is regulated," namely, the large set of conserved core components functioning in the animal's development and physiology. New regulation specifies new combinations, amounts, and functional states of those components to act at particular times and places in the animal. And fourth, the altered combinations, amounts, and states of the conserved components function to develop and operate a new trait on which selection acts. Of course the entire process is repeated in successive rounds of phenotypic variation and selection in an evolving trait.

The theory implies that new traits contain very little that is new in the way of functional components, whereas regulatory change is crucial. However, is a prohibitive number of regulatory changes needed to express thousands of genes at the new place and time of the new trait, and to operate thousands of encoded gene products (proteins and RNAs) at specific rates and in specific states? What quantity and quality of regulatory changes are needed? In answer, the theory of facilitated variation posits that core functional components, and the processes in which they serve, have special properties that greatly reduce the need for regulatory change, in ways that (*i*) reduce the number of necessary genetic changes, (*ii*) increase the variety of regulatory targets for change, (*iii*) reduce the amount of lethality due to genetic change, and (*iv*) increase the amount of genetic variation carried in the population. All of these effects facilitate the generation of viable phenotypic variation by regulatory change, and therefore we call it a theory of facilitated variation.

We will address three points of the proposals. What are the conserved core components and processes, what are their special properties that facilitate the generation of phenotypic variation by regulatory change, and what, in turn, are the regulatory innovations that have facilitated the use of core processes?

Conserved Core Components: Raw Material of Phenotypic Variation

These components generate and operate the animal's phenotype. Most are conserved across diverse phyla of the animal kingdom. Most operate in multicomponent processes that we call "conserved core processes." They comprise an enormous toolkit, and the genes encoding them comprise the majority of the genetic repertoire of the animal. They have changed very little in the course of animal evolution since the Cambrian, even though animal anatomy and physiology have changed. These conserved functional components comprise that which is regulated in the animal; regulation of them has changed in animal evolution.

To indicate their diverse indispensable contributions to the phenotype, we enumerate core processes in Table 1, associating each with one of four major episodes of pre-Cambrian functional innovation (mostly protein evolution). These biochemical, molecular genetic, cell biological, physiological, and developmental components (which fill the textbooks of these fields) were carried forward, unchanged, in all bilateral animals. This, we argue, was such a powerful and versatile toolkit that post-Cambrian animals could largely omit further functional innovation at the gene product level (protein and functional RNA evolution) and instead exploit regulatory innovation to diversify anatomy, physiology, and development. What is remarkable about the processes, as a large set, is that they can be used in so many contexts

toward so many ends. They define the envelope of possibilities of what regulatory change can achieve.

Parenthetically, though, some core components and processes have admittedly evolved since the Cambrian, and these, too, have become conserved. Appendage and limb formation (arthropods and tetrapods, respectively) would be developmental examples. These complex processes are, we argue, combinations of different conserved core processes linked in new regulatory configurations, conserved in their entirety. Others appear to entail protein evolution and new functions combined with old conserved processes, such as the SCPP proteins of bone formation, or keratins of hair and skin cells, or various myelin proteins of glial cells, or neural crest cells, or the adaptive immune system, all evolving in early vertebrates. These entail significant additions to the toolkit. And of course, protein evolution was very important in the four episodes of pre-Cambrian innovation described previously. For the most part, though, animals since the Cambrian have repeatedly reused the processes and components that had been evolved long beforehand to generate novel traits of anatomy and physiology.

Recent genome analysis has brought quantification to the impressions about conservation. More than 80 metazoan genomes have now been sequenced, and a typical case is the mouse (8). Of its total set of gene sequences, 23% are shared with prokaryotes, a further 29% are shared with non-animal eukaryotes (protists, fungi, and plants), and a further 27% are shared with nonchordate animals. Thus, 79% of mouse genes retain pre-Cambrian sequences. Reciprocally stated, only 21% of its functional components are unique to chordates, much less vertebrates, mammals, or mice. Such DNA sequence conservation among life forms conveniently allows the rapid identification of genes in new genomes by equating them with proteins or RNAs of other animals or yeast or bacteria where their function has been elucidated. As examples, the actins and β-tubulins of yeast and humans are 91% and 86% identical in amino acid sequence, respectively, and the otoferlins (a sensory cilium protein) of human hearing and *Drosophila* sensilla are 80% identical.

A complementary finding of genomics is the less-than-expected number of genes in animal genomes compared with bacteria and single-celled eukaryotes. The gene range from sea anemone (*Nematostella*) to human is 20–25,000 (D. Rokhsar, personal communication), with some exceptions reflecting gene loss (honey bee, 10,000; *Drosophila*, 13,600). These numbers are but two to five times the inventory of *Escherichia coli* (4,600) or yeast (6,400), even though animals seem much more complex in their anatomy and physiology. One way out of the seeming paradox both of an embarrassingly small gene number in animals and of the widespread sharing of gene sequences with other organisms is combinatorics (9, 10), the use of subsets of the same components in different combinations to get different outcomes, an interpretation we favor.

Why are such sequences conserved? All functioning proteins have specialized surface sites for precise interactions. At these sites, nonsynonymous amino acid substitutions are almost always detrimental to function and are eliminated by purifying selection, whereas synonymous substitutions are not (neutral or nearly neutral DNA changes), indicating that the conserved genes did undergo sequence change, like other DNA regions. For evolution, this deep conservation overwhelmingly documents the descent of animals from ancestors and has helped clarify phylogenetic relationships.

Functional conservation might seem to constrain phenotypic change because most sequence changes of those DNA regions encoding functional proteins and RNAs are lethal. (Note that the regulatory parts of proteins and RNAs are, we think, more changeable.) These DNA regions are effectively excluded from the list of targets at which genetic change could generate viable selectable phenotypic variation. They just cannot be tinkered with. Was evolution impeded by this vast functional conservation? We suggest that so much gene sequence is precluded from viable change that we should even revise our question about phenotypic variation to ask: what are the special properties of animals' phenotypes that allow phenotypic variation to be generated in seemingly copious amounts and great anatomical and physiological variety? These conserved processes have, we think, facilitated or deconstrained evolution because of their special properties of robustness and adaptability, their modularity and compartmentalization, their capacity for weak regulatory linkage, and their exploratory behavior. These properties make regulatory change efficacious and phenotypic variation copious and varied. We subsequently consider these properties and their consequences for regulation.

Weak Regulatory Linkage

Linkage, which denotes the connecting of processes to each other or to particular conditions, is central to our theory because different core processes must become linked, by regulatory means, in different combinations, and operated in different amounts, states, times, and places for the generation of new anatomical and physiological traits. Regulatory linkage pervades development and physiology. In general, a regulatory signal or input from one process or condition impinges on another process, which gives a response or output. The two are linked. Can regulatory linkages be made and changed easily, or do they require multiple complex instructions and precise stereochemical complementarity of the input and output? We argue that conserved core processes have a special capacity for weak regulatory linkage (4, 10), which reduces such demands and therefore facilitates the generation of phenotypic variation. In defining weak regulatory linkage, we stress two points: (*i*) the signal input and response output interact indirectly through an intermediate agency and hence do not require stereochemical complementarity to each other, and (*ii*) the output can be much more complex than the regulatory input because it has been previously built into the core process, independent of the nature of the signal. Although the signal seems superficially to control the response, it invariably turns out that the responding core process can produce the output by itself but inhibits itself from doing so. This self-regulation is built into the process. The signal, then, merely interferes with the self-inhibition (the intermediate agency), thus releasing the output, which may be much more complex than the signal and needs little instruction from it. In evolution, the signal is selectable just for its regulatory value, without regard to its chemical relationship to the response or to its instructive capacity. The regulatory input and functional output need not coevolve. Conceptually, the alternative is "strong linkage" (e.g., cofactors and substrates), which, we argue, requires more complex, precise, informative, and direct interactions from the input to make a process give a particular output. Constraint to change would be greater; more genetic change seems required.

Allosteric proteins, also known as switch proteins, are the simplest examples. These pervade metabolism, signal transduction pathways, neuronal excitation, transcriptional regulation, and physiology (e.g., hemoglobin). The protein's intrinsic activity is self-inhibited by a change of conformation of the protein and/or repacking of its subunits. The protein spontaneously switches between on and off states of activity but, on its own, strongly favors the off state. Regulatory agents select one or the other state by binding more strongly to it. This binding stabilizes the state, increasing its frequency in the protein population. Any regulator binding better to the on-state is an activator; any binding better to the off-state is an inhibitor. It is important to note that activity and inactivity are built into the protein, without instruction from the regulator, which only performs a state

selection. Control of the protein is minimal. The regulator does not bind near the functional sites of the protein and need not be structurally compatible with them. They do not coevolve. Regulatory linkages can evolve with little constraint.

Neuronal transmission is a more complex two-state example, a physiological process comprising several core processes. The neuron connects inputs (received neurotransmitters) to distant outputs (the secretion of other neurotransmitters). To do this, the neuron generates two states, resting and active, which differ in their membrane potential. The resting state with a more negative potential blocks the secretion of neurotransmitters. The active state with a less negative potential permits secretion. The received neurotransmitter initiates a local opening of allosteric ion channels, and local depolarization, at one end of the resting neuron. Weak linkage is provided by the propagated change of membrane potential, activating the entire neuron. When the other end becomes activated, it initiates secretion. The input (receptors and ion channels) is largely independent of the output (the secretory mechanism), connected only by the propagated depolarization. Receptors and ion channels can be installed or removed without reconfiguring secretion, membrane polarization, or impulse propagation, which are all conserved. They do not have to coevolve. In this case weak linkage has probably facilitated the evolution of the large variety of receptors, ion channels, and nerve cell types.

A still more complex example of weak linkage is embryonic induction, a developmental process first described in 1924 by Spemann and Mangold (11). Here a small group of cells, the "organizer," induces the development of the central nervous system in nearby cells of the rest of the vertebrate embryo. At the time, it was thought this induction must entail detailed instructions to the responding cells. A surprising discovery of the past decade is that the organizer acts by secreting a few inhibitors (antagonists) that do not even bind to the responding cells (12). Instead, they antagonize an inhibitory signal secreted and received by the nearby cells in a self-inhibitory circuit to block their development of the nervous system. The organizer, via its antagonist, disrupts the self-inhibition, and neurogenesis commences. Thus, a simple signal, which can easily be moved, replaced, or modulated, regulates the time, place, and amount of the very complex developmental response. The ease with which simple signals can entrain complex processes reflects the capacity of core processes to engage in weak regulatory linkage.

Finally, the action of enhancer binding proteins in eliciting or repressing transcription (a complex specific output) is an excellent example of weak linkage. Transcription factors bind to the genome and mobilize enzymes that modify chromatin; the factors do not directly contact the core transcriptional machinery and play no role in transcript elongation, only in the initiation decision. Because of weak linkage, cis-regulatory DNA sites at which transcription factors bind can be far from the transcription start site, in either orientation, and composed of numerous independently acting regions (13).

Weak regulatory linkage is important in developmental plasticity, which West-Eberhard has persuasively argued is a frequent substrate for heritable regulatory cooption (14). This plasticity entails the choosing of alternative developmental pathways according to environmental inputs. Examples include male–female differences, learning, and alternate jaw structures. In her view, if the capacity to develop large phenotypic differences already exists in the organism as self-inhibited alternate states, and these can be elicited by simple signals (weak linkage), then large evolutionary steps can be made with a modicum of genetic change. In such cases, the distinction blurs between evolutionary gradualism and saltation (the generation of significant traits by single mutations). As an example, sex in some vertebrates (fish and reptiles) is determined environmentally (temperature, crowding, or social interactions) but in others, heritably (sex chromosomes). The underlying mechanisms for sex determination are similar in all vertebrates. It is just that an environmental stimulus (acting via weak linkage) has been replaced by a genetic one in the sex chromosome case. Neither provides much information about the outcome but just acts on the conserved switch.

To summarize, the relevant point of these examples is that regulatory change is easily effected when conserved core processes have an inherent capacity for weak regulatory linkage, that is, when switch-like behavior and alternative states of function are already built into them. The regulator need not inform the response or be stereochemically compatible with it. Regulation does not need to coevolve with the functional response. The requirements for regulation and regulatory change are reduced.

Exploratory Processes

As the name implies, some conserved core processes appear to search and find targets in large spaces or molecular populations. Specific connections are eventually made between the source and target. These processes display great robustness and adaptability and, we think, have been very important in the evolution of complex animal anatomy and physiology. Examples include the formation of microtubule structures, the connecting of axons and target organs in development, synapse elimination, muscle patterning, vasculogenesis, vertebrate adaptive immunity, and even behavioral strategies like ant foraging. All are based on physiological variation and selection. In the variation step, the core process generates not just two output states, but an enormous number, often at random and at great energetic expense. In the selective step, separate agents stabilize one or a few outputs, and the rest disappear. Although that agent seems to signal the distant process to direct outputs to it, it actually only selects locally via weak linkage among the many outputs independently generated by the process. Components of the variation and selection steps of the process are highly conserved.

Microtubules, for example, adopt vastly different spatial arrays in different cells. First, the tips of numerous microtubule polymers grow outward from a nucleation center, in random directions (the variation event). Each polymer is unstable and, after a short time, by chance, shrinks back from the tip (15). They probe all regions of the cell in a futile cycle of outgrowth and shrinkage. If one by chance encounters a stabilizing agent at the cell periphery, its end is trapped, preventing shrinkage (the selection event). The entire length of microtubule leading to the agent is preserved. As more microtubules are selectively stabilized in one location, the cell's anatomy becomes polarized. This process is very adaptable and robust, providing microtubules no matter where stabilizers are located. It can therefore accommodate to placement errors or changing needs of the cell and can serve diverse roles, as in cilia, axons, and the mitotic spindle. Although the process of outgrowth and shrinkage is strongly conserved, and hence internally constrained in its own change, it generates diverse arrays each time it is used. In any particular cell, most outcomes are wasted, but they can be put to new uses in evolution simply by other cells' placing selective agents in new locations.

Wiring of the nervous system also draws heavily on exploratory processes. Excess axons extend from the central nervous system and randomly explore the body's periphery. Some accidentally hit target organs, such as muscles, and receive a dose of stabilizing protein (nerve growth factor); they persist, while others, failing contact, shrink back to the central nervous system.

Robustness and Adaptability

Weak regulatory linkage, state selection, and exploratory behavior underlie the robustness and adaptability of conserved core processes, that is, their capacity to produce functional (viable) out-

comes despite physiological, developmental, environmental, or even evolutionary change. Robustness implies that a process remains the same because of tolerance or resistance to changing conditions, and adaptability implies that a process changes with the conditions in ways still to achieve the objective. Related to such properties, several authors have discussed the positive role of phenotypic plasticity in evolution (14, 16); we feel that plasticity reflects the robustness and adaptability of core processes linked in complex assemblies. Robustness and adaptability are essential to the kind of evolution we have described, wherein core processes are used in different combinations, amounts, and states to produce new traits. They strongly reduce the requirements for regulatory change, and hence genetic change, and increase the frequency of viable phenotypic variations.

Adaptable robust processes can support nonlethal phenotypic variation in other processes, a situation called "accommodation" by West-Eberhard (14). A specific example is the evolution of the tetrapod forelimb to a bird or bat wing. Not only did the length and thickness of bones change, but also the associated musculature, nerve connections, and vasculature. Did many regulatory changes occur in parallel, coordinated by selection, to achieve the coevolution of all these tissues in the limb evolving to a wing? The answer comes from studies of limb development showing that muscle, nerve, and vascular founder cells originate in the embryonic trunk and migrate into the developing limb bud, which initially contains only bone and dermis precursors. Muscle precursors are adaptable; they receive signals from developing dermis and bone (17) and take positions relative to them, wherever they are. Then, as noted previously, axons in large numbers extend into the bud from the nerve cord; some fortuitously contact muscle targets and are stabilized, and the rest shrink back. Finally, vascular progenitors enter. Wherever limb cells are hypoxic, they secrete signals that trigger nearby blood vessels to grow into their vicinity (18). This self-regulating vasculogenesis operates not just in the limb but throughout the body, accommodating to growing tissues, to exceptional demands such as pregnancy, and alas to growing tumors. The adaptability and robustness of normal muscle, nerve, and vascular development have significant implications for evolution, for these processes accommodate to evolutionary change as well. In the case of the evolving wing, if bones undergo regulatory change (driven by genetic change) in length and thickness, the muscles, nerves and vasculature will accommodate to those changes without requiring independent regulatory change. Coevolution is avoided. Selection does not have to coordinate multiple independently varying parts. Hence, less genetic change is needed, lethality is reduced, larger phenotypic changes are viable, and phenotypic variation is facilitated.

Finally, as Schmalhausen, Waddington, and others (19–21) have argued, physiological and developmental robustness reduces lethality because of undirected genetic variation. Less genetic variation is eliminated from the population, leaving it available for new trials of regulatory combinations and effects.

Favorable Sources and Paths of Phenotypic Change

Several authors tried in the past to connect long-term evolutionary change to short-term physiological change. As well known, Lamarck postulated that animals undergo anatomical and physiological changes in response to the environment, and then their offspring inherit these acquired characteristics. Darwin first conceived of variation as undirected and small with respect to selective conditions but later drifted toward Lamarck in thinking that as the organism responds to conditions, it furnishes the gametes with information enhancing the next generation's response. In a 30-year period of confusion after Darwin, various evolutionists made internally driven phenotypic variation the creative factor in evolutionary change (e.g., orthogenesis and macromutation), even dismissing selection. The Modern Synthesis of the 1930s to 1950s dispelled such ideas about organism-directed phenotypic variation by combining Darwin's original hypothesis with new insights from transmission genetics, population genetics, and paleontology. Selection was restored to its central place.

Parallel to the Modern Synthesis, less known ideas did succeed in connecting long-term and short-term phenotypic variation without requiring an inheritance of acquired characteristics. Some of these premolecular ideas relate to recent proposals about the role of the organism in variation (16, 22) and to our proposals of facilitated variation. Baldwin in 1896 and 1902 reconciled aspects of Lamarck's and Darwin's proposals in what is now called the Baldwin effect (23, 24). Accordingly, if an animal makes short-term physiological or behavioral adaptations to the environment, and then the conditions persist, these adaptations remain under selection, because at the adaptive limit they only provide marginal survival. They can become stabilized and extended by genetic change and hence become heritable traits. For Baldwin, adaptability of the animal's physiology and development is the source and path of evolutionary change.

Schmalhausen (19) extended these ideas in the 1940s to include all nonlethal phenotypic changes of an organism that can be evoked by the environment, some adaptive to the evocative condition and others not ("morphoses" he called them), some reversible (physiological) and others not (developmental). He called this enormous range of phenotypes, which are achievable without genetic change, the animal's "norm of reaction" to the environment. Once evoked, any of these traits could, under selective conditions, be stabilized and enhanced by genetic change, which he anticipated to be of a regulatory nature. Thereafter, the trait's expression in the new conditions would be heritable. For physiological and developmental adaptations, evocative and selective conditions were the same. For morphoses, a selective condition would fortuitously overlap the evocative condition.

Waddington independently developed similar ideas in the 1940s and 1950s under the name of "genetic assimilation." He evoked phenotypic changes in *Drosophila* by ether, heat, or salt treatment and then, after 21 generations of treatment and selection, obtained flies that heritably exhibited new phenotypes without treatment (20). Interestingly, the heritable fixation of the new traits was polygenic and arose only in genetically heterogeneous (non-inbred) populations, through repeated mating at the adaptive limit. Seemingly, the original population contained numerous variants of small effect, each too small for the full trait, and then, as the marginal population mated for 21 generations, various small regulatory differences combined to the full trait (25). Recently, Rutherford, Lindquist, and colleagues (26) used heat, small-molecule inhibitors, and stop-codon suppressors to evoke a wide variety of new phenotypes (in interpretable ways) in *Drosophila*, plants, and yeast and recognized the latent variation of these phenotypic responses. Their phenotypes, too, could then be stabilized by genetic change under selective conditions, imposed by the experimentalist.

A major implication about phenotypic variation from these studies and ideas is that when novelty of some kinds is achieved in the course of variation and selection, rather little is really new; most components and regulatory linkages of the trait were already there. Novelty rests on small regulatory changes just stabilizing and enhancing an already extant physiological or developmental adaptation or evocable aberration. These were early attempts to restore the organism's present phenotype to the variation-generating process while still requiring genetic change. They also emphasize phenotypic plasticity (robustness, adaptability) as providing favorable sources and paths of evolutionary change, requiring few genetic changes. Such interpretations seem particularly suited to directional selections on physiological functions.

Recent advances in cell and developmental biology raise other possibilities for sources and paths of phenotypic variation. As noted previously, cellular adaptations occur repeatedly during development. Embryonic cells usually possess two or more developmental options under the control of a switch-like circuit. Via weak regulatory linkage. they respond to signals from neighboring cells, choosing one or the other option. At different times and places in the embryo, cells have different response options. If the adaptive states of embryonic cells are enumerated (states of gene expression, proliferation, secretion, shape, and signaling), the number is enormous. We suggest that this developmental cellular plasticity, which is based on ensembles of core processes already linked in various regulatory ways, is a major cryptic source of evolutionary novelty by regulatory stabilization. Such plasticity is, we think, rarely evocable by environmental conditions and hence would be omitted from the Baldwin effect.

Neural crest cells of vertebrates are a compelling example. These originate at the edge of the neural plate in early vertebrate development and migrate ventrally in the embryonic body, exploring numerous settlement sites having different regulatory signals. The cells possess many differentiation options (states), nearly unlimited powers of proliferation, and wide receptivity to local signals. Just within the head, they account for teeth, skull bones, the elephant's trunk, the narwhal's unicorn-like tooth, deer antlers, and probably the head shield of ceratopsian dinosaurs. These may all be but minor regulatory perturbations on neural crest cell adaptability, provided at the settlement site (time, place, amount of local signals). Sewell Wright (27) was prescient, we think, when he noted in 1931, "The older writers on evolution were often staggered by the seeming necessity of accounting for the evolution of fine details . . . for example, the fine structure of all of the bones . . . structure is never inherited as such, but merely types of adaptive cell behavior which lead to particular types of structure under particular conditions."

Although we concur that externally directed phenotypic plasticities are a rich source of variations for regulatory stabilization, we add to it the richer source of internally directed cellular developmental adaptations. The latter class would not be evoked by the environment and then stabilized, but stabilized directly by regulatory change driven by genetic variation.

Compartmentation

Thus far we have discussed how conserved core processes facilitate regulatory change, but we should also discuss how various regulatory processes, evolved in pre-Cambrian animals, have facilitated the use of core processes in different combinations, amounts, and states, while decreasing their chances of interference (pleiotropy). Spatial compartmentation of transcriptional regulation and cell–cell signaling is one of these.

In bilateral metazoa, the body of the mid-stage embryo, sometimes called the phylotypic stage of development, becomes divided into a regulatory grid or map of small compartments, each uniquely defined by its expression of one or a few selector genes encoding transcription factors or signaling molecules. The insect embryo at this stage contains ≈100 contiguous compartments, and the vertebrate embryo contains perhaps 200. The map is highly conserved within a phylum, and the stage is called phylotypic because embryos of all classes of the phylum then look most similar. Thereafter, selector genes of a compartment specify the anatomy and physiology to be developed within it; they "select" other genes, some encoding regulators and some encoding core process components, to be expressed or repressed in their compartment, thereby combining and customizing core processes for local usage. Different combinations, amounts, and states of core processes can be engaged in parallel in numerous regions of the embryo (28, 29). Conflicting processes such as cell death and proliferation can be run separately without interference.

One example of compartmentation is found in developing vertebrae, all of which contain bone-forming cells. In thoracic vertebrae they also form ribs, whereas in the cervical vertebrae they do not. Despite their equivalence as bone-forming cells, they differ, as shown by transplantation experiments (30), solely because they arose along the dorsal midline in different compartments expressing different Hox genes. Similarly, *Drosophila* has a single developmental process for forming appendages; in the thorax it produces a leg, but in the head it produces biting mouthparts, because of different regulators introduced by different selector genes (6, 31). Likewise, the forelimbs and hind limbs of vertebrates differ because of compartment-specific regulatory differences (Hox and Tbx genes).

Compartmentation facilitates the generation of phenotypic variation; that in one compartment does not constrain that in another (31). Regulatory specification occurs independently and in parallel in different compartments. Also, we think that the compartment map deconstrains development preceding the phylotypic stage, when it first appears. The single-celled egg, we suggest, develops the compartment map by a robust adaptable process requiring little regulatory input. Thereby, the egg is freed to evolve fitness-enhancing diversifications of size, shape, nutrient provision, and gastrulation, as happened repeatedly in chordates and arthropods. After the phylotypic stage, as noted previously, members of different classes and families diversify their anatomies and physiologies, depending on which processes and regulation each compartment selects. The location of a conserved process (the compartment map) between diversified processes has been called the "bowtie effect" by Csete and Doyle (32), who discuss its design benefits.

Other forms of regulatory compartmentation also facilitate diverse combinatorial uses of the gene repertoire while reducing pleiotropic interference. Each of the several hundred differentiated cell types of vertebrates is probably controlled by a few transcription factors and signaling proteins encoded by master regulatory genes, which select the expression of other regulatory genes and core processes of that cell's phenotype. In the temporal dimension, developmental stages such as the embryo, larva, and adult are sometimes compartmentalized by expressed heterochronic genes (33) that select stage-specific target genes, and in sexual dimorphism, target genes are selectively expressed in each sex.

Experimental Evidence for Facilitated Variation

To summarize, we argue that robustness, adaptability, modularity, capacity for weak regulatory linkage, exploratory behavior, and state selection of the conserved core processes, as well as the regulatory compartmentation of the conserved core processes, are key properties of the animal's phenotype that facilitate the generation of anatomical and physiological variation by regulatory change, which ultimately requires genetic change to be heritable. These special properties reduce the number of genetic changes needed for phenotypic change, increase the number of targets for regulatory change, reduce lethality, and increase genetic variation retained in the population. Although the core processes are constrained in their own change of function, they deconstrain regulatory change.

Is this a testable hypothesis or merely a post hoc rationalization? To begin with, we should say that the theory emphasizes the targets of change and their consequences for phenotype, not the paths of change, although we especially like the plasticity-based paths because of what they say about targets and reuse of components. Basically, we accept any kind of regulatory change, arising by any path of genetic change, as long as it affects the combinations, amounts, states, times, and places of conserved core processes. Included would be the neo-Darwinian possibility of a rare, favorable, nonlethal, penetrant mutation that is selected to fixation of a new phenotype, and also included would be the Baldwin possibility of physiological adaptation at first

without genetic change (in response to environmental change), followed by regulatory changes (via new allele combinations) enhancing and fixing a new phenotype. In both cases, genetic change results in regulatory change, which modifies the use of the conserved core processes.

The theory predicts that developmental biologists will continue to find (*i*) more examples of core processes used in diverse developmental and physiological traits in different combinations, amounts, and states, and (*ii*) in each new case a few small regulatory changes sufficing to redeploy core processes, which are themselves robust and adaptable. When introduced experimentally, such regulatory changes should significantly alter the phenotype, and other processes should accommodate to the directly altered ones, giving viable outcomes. Furthermore, it predicts that, as comparative experimental studies uncover the history of evolutionary innovation in animals, regulatory types of changes will predominate. Indeed, as is already clear, altered cis-regulation of gene expression and altered production of secreted signals lie behind specific phenotypic changes in stickleback fish and *Drosophila* (34–36).

A recent example of bone morphogenetic protein (Bmp) and calmodulin signaling supports facilitated variation via robust adaptable processes. As described in the Introduction, Darwin noted the rapid divergence of beak morphologies by Galapagos finches. If we think mostly about selection and not phenotypic variation, we might imagine that selection acted repeatedly on many small changes occurring independently in the upper and lower beaks, adjacent skull, and head muscles to coordinate and order them into viable intermediate beaks throughout divergence. Many regulatory changes and many selections would be needed for this detailed coevolution of parts. Recent results, however, make a different impression. Tabin's group has compared two Galapagos finches, one with a large nutcracker-like beak and another with a small forceps-like beak (37). In beak development, neural crest cells migrate from the neural plate to five primordia around the mouth. The primordia of the large-beaked finch express Bmp earlier and at higher levels than do those of the small-beaked finch. To test the importance of this difference, they introduced Bmp protein into the primordia of a chicken embryo, which normally develops a small pointed beak. The experimental chick developed a deep, broad beak, like the large-beaked finch. The beak was not monstrous; its parts fit together and properly adjoined the head. Recently the same group demonstrated that elevated levels of calmodulin, a ubiquitous calcium signaling protein, correlate with increased beak length, and experimental increases of this protein in the developing chick beak caused coordinated increases in beak length (38). Thus, two highly conserved factors quantitatively control much of the overall anatomy of the beak and adjacent head, producing a functional structure. Much coordination of parts is inherent in beak development; selection need not direct a detailed coevolution of parts; larger beak variations may be viable and selectable. Similarly the exuberant radiation of jaws in cichlid fishes of Lake Malawi is now attributed to changes at a small number of quantitative trait loci (39), including for Bmp. These results imply quantitative adjustments on robust, adaptable processes due to a few regulatory changes rather than many small independent changes coordinated by repeated selections.

A final feature deserves mention. Regulatory changes of the level of Bmp in the finch beak are in principle achievable in many ways, not only through altered transcription of the *bmp* gene (i.e., cis-regulation), or translation of the mRNA, or secretion, post-translational modification, proteolytic processing, and breakdown of the protein. The levels of Bmp receptors could also be altered, as could the levels of any of several agonists and antagonists. Regulatory targets are many, yet all change Bmp signaling strength. Regulatory modification of the strength of Bmp or calmodulin signaling within one spatial compartment may have sufficed to achieve functional selectable changes in beak shape in a few steps. Other conserved processes also have multiple targets for regulatory change.

Facilitated Variation and Evolution

Although recent insights in developmental biology and physiology deepen the understanding of variation, they do not undermine evolutionary theory. Laws of variation begin to emerge, such as regulatory change as the main target of genetic change, the means to minimize the number and complexity of regulatory changes, and the regulatory redeployment of conserved components and processes to give phenotypic variations and selected traits. Regulatory change acts on the repertoire of unchanging core processes to select subsets, which are then externally selected upon. The burden of creativity in evolution, down to minute details, does not rest on selection alone. Through its ancient repertoire of core processes, the current phenotype of the animal determines the kind, amount, and viability of phenotypic variation the animal can produce in response to regulatory change. Thanks to the nature of the processes, the range of possible anatomical and physiological variations is enormous, and many are likely nonlethal, in part simply because the processes have been providing "useful" function since pre-Cambrian times. Phenotypic plasticities, both those evokable by environmental change and those developmental adaptabilities not evocable, are rich sources and favored paths of variation requiring little regulatory change.

These views are not at all Lamarckian, nor does facilitated phenotypic variation require selection for future good. Such facilitation arose, we think, as a by-product of the evolution of the special properties of the core processes, namely, of their robustness, adaptability, modularity, exploratory behavior, and capacity for weak regulatory linkage. These properties were probably selected at the level of the individual, simply for their capacity to make core processes work effectively under fluctuating external and internal conditions (4, 10). In this way, the same molecular features that facilitate physiological and developmental change in an organism's lifetime also facilitate evolutionary change in the long run, as regulatory changes become genetically fixed.

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