



SYMPOSIUM

Integrator Networks: Illuminating the Black Box Linking Genotype and Phenotype

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Synopsis Emerging concepts in developmental biology, such as facilitated variation and dynamical patterning modules, address a major shortcoming of the Modern Synthesis in Biology: how genotypic variation is transduced into functional yet diverse phenotypic variation. Still, we lack a theory to explain how variation at the cellular and tissue level is coordinated into variation at the whole-organism level, especially as priority of cellular and tissue functions change over an individual's lifetime and are influenced by environmental variation. Here, we propose that interactions among a limited subset of physiological factors that we call, integrators, regulate most phenotypic variation at the organismal level. Integrators are unique among physiological factors in that they have the propensity to coordinate the expression of conserved gene modules of most types of tissues because they participate as nodes in a hierarchical network. In other words, integrator networks impose physiological epistasis, meaning that whole-organism phenotypic responses will be influenced by previous experiences, current environmental conditions, and fitness priorities as encoded by individual integrators. Below, we provide examples of how integrator networks are responsible for both profound and irreversible phenotypic changes (i.e., metamorphosis, sexual differentiation) as well as subtler, transient (e.g., pelage color, seasonal fluctuations in lymphoid and reproductive tissues) variation. The goal of this article is not to describe completely how integrator networks function, but to stimulate discussion about the role of physiology in linking genetic to phenotypic variation. To generate useful data sets for understanding integrator networks and to inform whole-organism physiology generally, we describe several useful tools including vector-field editing, response-surface regression, and experiments of life-table responses. We then close by highlighting some implications of integrator networks for conservation and biomedicine.

Introduction

In recent years, phenotypic plasticity, the expression of different traits or trait values in different environments by the same genotype, has been well-documented and found to influence ecological and evolutionary processes in many species (Huey and Kingsolver 1989; Pigliucci 2001; West-Eberhard 2003; Ghilambor et al. 2007). Moreover, a

heightened awareness of the scope of phenotypic plasticity has led to calls for extensions to the foundations of evolutionary biology (Pigliucci 2007), in extreme cases re-thinking the roles of genes in the evolutionary process (i.e., as followers instead of drivers of evolutionary change) (West-Eberhard 2003). Still, there is much to learn about the mechanisms of phenotypic plasticity namely how a given

genotype produces variable, but viable phenotypes (Nussey et al. 2007). Evolutionary developmental biologists have recently started to try to fill this void, recognizing that most organisms utilize similar core processes to carry out vital functions (Kirschner and Gerhart 1998; 2010). Bacteria, fungi, plants, and animals all utilize the same molecular tool kits and dynamic patterning modules to build cells, replicate DNA, and metabolize energy substrates (Newman 2010). By expressing conserved genetic modules contingent on context, facilitated phenotypic variation is achieved. Specifically, (1) phenotypic variation can be maximized for a given amount of genetic variation, (2) lethality of phenotypic variation can be minimized, and (3) expression of phenotypic variation can be matched appropriately to the environment, even environments never before experienced by the genome (Kirschner and Gerhart 2010).

Although ideas such as facilitated variation and dynamic patterning modules address key, missing components of the Modern Synthesis of Evolutionary Biology, they still lack some important elements, especially in regards to large organisms. First, they do not explicitly propose a mechanism whereby the diverse cells and tissues of multi-cellular organisms are coordinated to build a functional, behaving organism. For example, reproductive and lymphoid tissues are integral to individual fitness, but they provide different benefits and compete for limiting resources (Martin et al. 2008). An individual must have a mechanism in place to determine what resources are available and how to best allocate them among tissues (Bilbo and Nelson 2003; Bilbo et al. 2003; Pyter et al. 2005; Weil et al. 2006). Second, concepts in the previous paragraph lack the ecological perspective that is important in predicting how whole-organisms will respond to environmental variation. Phenotypic change is often determined via a cost–benefit analysis by individuals (Williams 1966): an organism living in a parasite-rich environment would likely experience the greatest fitness if immune activities were enhanced and reproduction suppressed, whereas an individual living in a parasite-poor habitat may invest little in immune defense at all (Piersma 1997; Buehler et al. 2008).

Here, we propose that networks of specific physiological factors, here called integrators (Supplementary Table S1), coordinate appropriate activation and use of gene modules and hence facilitate variation at the whole-organism level. Integrators (including but not limited to gibberellin, ecdysone, and glucocorticoids; see Supplementary Table S1) are unique among physiological factors because they respond to stimuli from both external

and internal environments and thus function as conduits through which the genotype, phenotype, and environment are linked. As organisms are closed-systems in regards to most physiological processes, all cells and tissues have the opportunity to be exposed to integrators. The potential to respond to integrators, therefore, allows adaptive phenotypic responses, in which distinct, and sometimes competing, pressures (e.g., predation, competition, risk of infection, time of year, availability of resources, and opportunity for reproduction) are addressed simultaneously. These outcomes are possible because of five key characteristics of integrators (Supplementary Tables S1 and S2): they (1) impact multiple types of tissue; (2) have persistent and transient effects; (3) are the endpoints of multi-step biochemical pathways and therefore are subject to upstream regulation (i.e., quality control); (4) encode information about an individual's past experiences, current condition, and the environment, in which it finds itself; and perhaps most importantly; (5) have reciprocal influences on each other, enabling them to act as a network.

We expect that reciprocal interactions among integrators and persistent changes in integrator network states are the main mediators of multi-trait phenotypes, including adaptations (Carroll 2005). Because selection pressures act on suites of correlated traits encoded by many genes (Reznick and Ghalambor 2001; Feder and Mitchell-Olds 2003), classic approaches have had difficulty explaining or predicting how gene expression is ultimately coordinated into appropriate phenotypic responses. In plants, for example, time of flowering is arguably one of the best-understood adaptations, and at least hundreds of genes are involved in the process. These tend to fall into four major functional categories: autonomous, gibberellin, photoperiod, and vernalization (Mouradov et al. 2002; Simpson and Dean 2002). Within these categories, mutant and transgenic studies have revealed that individual genes respond directly to specific environmental factors (i.e., the FRI gene product detects cold treatment, PHYA detects far red light, and PHYB, D, and E detect red light; Schmitt et al. 1999; Simpson and Dean 2002), and natural gene polymorphisms (i.e., FRI and FLC) result in dramatic phenotypic differences (Caicedo et al. 2004; Stinchcombe et al. 2004; Korves et al. 2007). However, how these individual genes coordinate with each other and incorporate environmental cues to appropriately regulate flowering time at the level of the whole plant remains unclear and relatively understudied (Mouradov et al. 2002).

For the remainder of this article, we describe how integrators might function in vertebrates and how we might study them. Our ideas have been motivated mostly by observational data, but also by studies that manipulated one or a few integrators and quantified consequences on others or on the whole phenotype (Supplementary Table S2). Although, many studies involve integrators (e.g., Supplementary Table S1), they rarely evaluate integrators as part of a network. We, therefore, highlight some well-studied examples and use these to generate ideas of how integrator networks function generally. We do not expect that we have identified all vertebrate integrators, nor do we expect that we have identified everything important about those we discuss. Thus, the last section of the paper describes some tools and approaches for learning more about integrator networks. At the least, we hope that this article generates discussion of comparable or alternative models for the assembly and coordination of whole-organism phenotypes.

How do integrators work?

Most physiological processes have four main elements (Medzhitov 2008): inducers, sensors, mediators, and effectors (Fig. 1A). In many systems, mediators are the hubs that determine cell output based on input of information from sensors. Mediators are typically of minor effect on the whole-organism though, influencing only downstream processes in a local physiological pathway. Integrators, however, are similar to mediators except that their effects can be broad, persistent, and impactful throughout an entire organism. In vertebrates, for example, cells and tissues that produce specific integrators also have receptors for other integrators (Supplementary Table S1); this trait is important as not all physiological factors can be perceived by all cells. Moreover, these receptors foster linkages among disparate physiological systems (e.g., immune, growth, reproductive, and digestive) and are what make integrators distinct from Medzhitov's mediators. For an individual, the response of one integrator to an environment is determined in part by the configuration of other integrators (Fig. 1B). Subsequently, small changes in the strengths, directions, or presence of connections among integrators (i.e., boundaries set by the genome) would determine the spaces that phenotypes can occupy (Crombach and Hogeweg 2008; Pigliucci 2008, 2009).

The complex phenotype the whole organism assumes is an outcome of the integrator network

configuration. The stronger the connections among integrators within the network, the less plastic the network will be to environmental perturbations (Siegal and Bergman 2002). Prior selection may canalize traits such that no variation in the influence of the integrator persists within the network. An obvious example is differentiation of secondary sex characteristics, a process impacted by androgens and estrogens in all vertebrates. Other examples include the impacts of glucocorticoids on coping style and/or personality (Meaney et al. 1996) and the effect of exposure to parasites on the propensity to respond aversively to allergens (Holt 1996). Furthermore, the stronger the connections between individual nodes, the greater are the impacts one integrator could have on another. The impacts of integrators within the network will be constrained by trade-offs borne when the functioning of a given integrator is in conflict with others [e.g., multiple costly systems functioning simultaneously (Stearns 1992; Monaghan and Nager 1997; Nijhout and Emlen 1998)]. At the organismal level, such trade-offs are manifest in patterns of allocation to growth, survival, and reproduction (Zera and Harshman 2001; Dufty et al. 2002; Ricklefs and Wikelski 2002). We expect that integrators could have evolved in part in ways that matches the phenotype to environmental conditions experienced previously by genotypes.

To illustrate integrator network interactions and their consequences for phenotypic variation, consider the following example (Fig. 1C). When an individual frog egg is fertilized, the initial relationships among integrators are determined by the genotype (and any transgenerational epigenetic effects), and serve much as a physiological character-identity network (Wagner 2007) so that the genotype and/or epigenotype (Richards et al. 2010) is what determines the possible phenotypes a genotype can take throughout life. Initially, many phenotypic outcomes are possible, but the specific outcome is contingent on experiences during development. For example, if a tadpole experiences stressful conditions prior to reproductive maturity, it should take a phenotypic trajectory determined predominantly by activation of stress-mediating integrators. However, the state of the rest of the integrator network will be impactful too (i.e., consider how male and female tadpoles might respond differently to stimuli given their unique life histories). If the stressor requires rapid development (e.g., drying of a pond) and resources are limited, up-regulating an integrator that increases growth rate would expedite metamorphosis, but also constrain the integrators that promote reproductive maturation and anti-parasite function. Conversely,

if a stressor requires an immune response, up-regulation of the integrators that help fight infection could alter the integrators that support metamorphosis, maturation, or both (Fig. 1D). In fact, certain tree frogs detect stimuli that inform them of threats as embryos (Gomez-Mestre et al. 2008) and such stimuli can initiate precocious hatching (Warkentin 1995). The decision to hatch early can have long-term consequences including alterations in growth rate, time to, and size at, metamorphosis, and anti-predator behavior in adulthood (Vonesh and Bolker 2005).

Vertebrate integrators

The strongest examples of vertebrate integrators are steroid hormones including some androgens and estrogens, and the glucocorticoids, as well as certain immune system cytokines, such as interleukin-1 β (IL-1 β), and other substances such as melatonin and leptin (Supplementary Table S1). These substances meet the criteria for integrators (Supplementary Table S1), exhibiting extensive and sometimes profound impacts on nontarget physiological processes (Supplementary Table S2). One proposed integrator, melatonin, is exceptional among the others because there is little evidence that other integrators affect it. This exception may be because of a lack of data or because melatonin encodes information about the external environment (i.e., day length), which organisms presumably use to reliably adjust the integrator network (Nelson et al. 2002). Similar exceptions may exist for integrators of condition or health, such as leptin and insulin. These integrators may restrict the configurations that the whole-integrator network can take, and thus be rather insensitive to other integrators. At present, it would be premature to claim that the factors we list in Supplementary Table S1 are the *only* vertebrate integrators; indeed, several other physiological factors exhibit some characteristics of integrators, and some of the substances listed might not qualify when studied in the manner we suggest below.

Unlike other hormones with small and local effects, the effects of integrators are pervasive and strong. Glucocorticoids (GCs), for example, are released due to inclement weather, attacks by predators, territorial conflicts, infection and injury, and other factors that require an emergency life-history state (Wingfield et al. 1998). During this emergency state, resources are shunted toward processes supporting immediate survival such as elevated blood

pressure and heart rate, and the release of glucose by tissues (Sapolsky et al. 2000), all processes thought to help an individual endure or recover from a stressor. However, GCs also inhibit release of pro-inflammatory cytokines, promote anti-inflammatory cytokine activities (Elenkov and Chrousos 2002), and inhibit secretion of reproductive hormones (Wingfield and Sapolsky 2003; Supplementary Table S2). Although such stress responses tend to resolve rapidly, sometimes they do not. In these cases, especially during particular periods of development, GCs can permanently shift an organism into a different phenotypic state than would have occurred in the absence of the stressor. Some of the strongest enduring effects include alterations in rates and timing of growth and metamorphosis in amphibians (Denver 2009), dispersal in lizards (De Fraipont et al. 2000), prenatal sex ratio in birds (Pike and Petrie 2006), and health throughout life (Chin et al. 2009). Effects are especially pervasive for rodents' immune functions (Shanks et al. 2000; Galic et al. 2009), particularly if activation of GCs happens well before maturation.

Of all of the enduring effects of GCs, the most profound might be their capacity to alter their own regulation. In mammals (Weaver et al. 2004), birds (Love et al. 2008), and amphibians (Denver 2009), stressors early in life alter the release and regulation of GCs throughout life. Such adjustment likely alters the dominance of GCs within the integrator network (Supplementary Table S2). Subsequently, contingent on early-life experience, individuals would become hypersensitive or hyposensitive to stressors, altering their morphology, physiology, and personality for the rest of their lives. Weaver et al. (2004) provided compelling evidence that pups that were licked and groomed more by their mothers had lower levels of methylation of one glucocorticoid receptor promoter than did pups that were licked and groomed less (Weaver et al. 2004). Low methylation of the GR promoter translated into increased expression of GR and less anxious behavior and reduced GC release to stressors in adulthood. What sets these results apart from a typical behavioral endocrinological study, and hence elevates GCs to a more important position than "just a hormone", is the potential evolutionary consequences of GCs if they act pervasively in this way. In this system, methylation status can be altered by maternal behavior and passed transgenerationally, which would impact the rate and extent of selection on GC-mediated phenotypic traits. If GCs have similar impacts in other vertebrates, individuals could exhibit and inherit extensive phenotypic variation without a change in the

DNA sequence of their genomes. Although space constraints prevent description of other examples of enduring integrator effects, a growing list suggests that epigenetic inheritance is probably underappreciated (Crews et al. 2007; Jirtle and Skinner 2007).

In contrast to GCs, the vertebrate integrators that have considerable evolutionary implications, but are least studied for their integrator roles are those involved in control of disease. Perhaps this lack of study persists because there are few whole-organism concepts regarding immune defense at all (Kopp and Medzhitov 2009). In spite of this lack of theory, some cytokines are implicated as integrators, namely interleukin (IL) 1- β . First, IL1- β , which is usually studied for its role in inflammation, can have enduring effects on response to infection. For example, individuals experiencing infections early in life respond more efficiently to the same infections in adulthood (Galic et al. 2009). Second, IL1- β functions can constrain and be constrained by other integrators; the attenuated immune responses in adulthood mentioned above are partly due to the actions of GCs, and IL1- β can dampen reproductive activities (i.e., androgen production via the testes) during infections (Turnbull and Rivier 1997). More recently, observations have suggested that other cytokine integrators may exist (e.g., IL-15), providing organisms with “memory of danger” (Noble 2009). Such integrators may be the long-sought progenitors of the “hygiene hypothesis” (Yazdanbakhsh et al. 2002), which proposes that disposition toward allergy in adulthood is due to exposure to parasites early in life.

Why do organisms use integrators?

The above conceptualization of integrators and integrator networks casts them more as constraints on, rather than facilitators of, variation. However, a major selective advantage of integrators was probably that they fostered evolvability of genotypes as well as stability and flexibility to phenotypes (Kirschner and Gerhart 2010). Metazoans are large conglomerations of cells organized into diverse organs and tissues that must mount coordinated responses to environmental fluctuations in order to survive and reproduce. Integrators may have initially been advantageous because they supported communication among different kinds of cells in much the same way that dynamic patterning modules for cell morphology were co-opted from cell differentiation pathways to create multi-cellular organisms from

unicellular ones (Newman 2010). Only later, when organisms grew in size or complexity, did integrators take on their current roles.

We hypothesize that integrators were selected to maintain their current function because they enabled individuals to utilize core molecular processes (Kirschner and Gerhart 1998) cohesively (i.e., symmorphosis) (Weibel et al. 1991). Much as expression of a conserved set of spatially arrayed genes ensures a phylotypic body plan (Woods 2009), integrators probably ensure that phenotypes are comprised of modules that maximize fitness in different contexts (Schlichting and Smith 2002). Indeed, a major effect of integrators is to shift the homeostatic set-points of physiological systems (i.e., rheostasis and allostasis). Although these effects have long been known, an underappreciated observation is that the magnitude and type of shift is impacted by the environment, including the states of other integrators (Mrosovsky 1990; McEwen and Wingfield 2003; Romero et al. 2009; Woods 2009). Moreover, phenotypic plasticity is likely important for the success of native populations enduring environmental changes (Nicotra et al. 2010) as well as of populations undergoing expansions of geographic range (Losos et al. 2004; Phillips and Shine 2006; Duckworth and Badyaev 2007). Yet with few exceptions (Yeh and Price 2004; Duckworth 2008), plastic responses in behavior and morphology have not been linked to the specific physiological processes that underlie them. We suspect that in many cases integrator networks facilitate adaptive phenotypic plasticity.

Integrators have probably been important in diversification too because they would tend to generate coordinated phenotypic variants, allowing for more efficient exploration of adaptive landscapes than could occur if exploration were independent of context (Pigliucci 2010). Many studies demonstrate that integrators generate functional phenotypic novelties by influencing the timing of important developmental transitions (i.e., heterochrony) (Wada 2008) or revealing hidden regions of reaction norms under novel environmental conditions (Schlichting and Smith 2002). These pathways (heterochrony and plasticity), are common sources of novelty in metazoans, including patterns of pigmentation in butterfly wings (Brakefield et al. 1996) and paedomorphosis in some salamanders (Reilly et al. 1997). Although the roles of integrator networks in such responses are not well studied, many are implicated. Nevertheless, novel phenotypic variation would often be adaptive in heterogeneous environments (Windig et al. 2004), reducing directional selection and/or

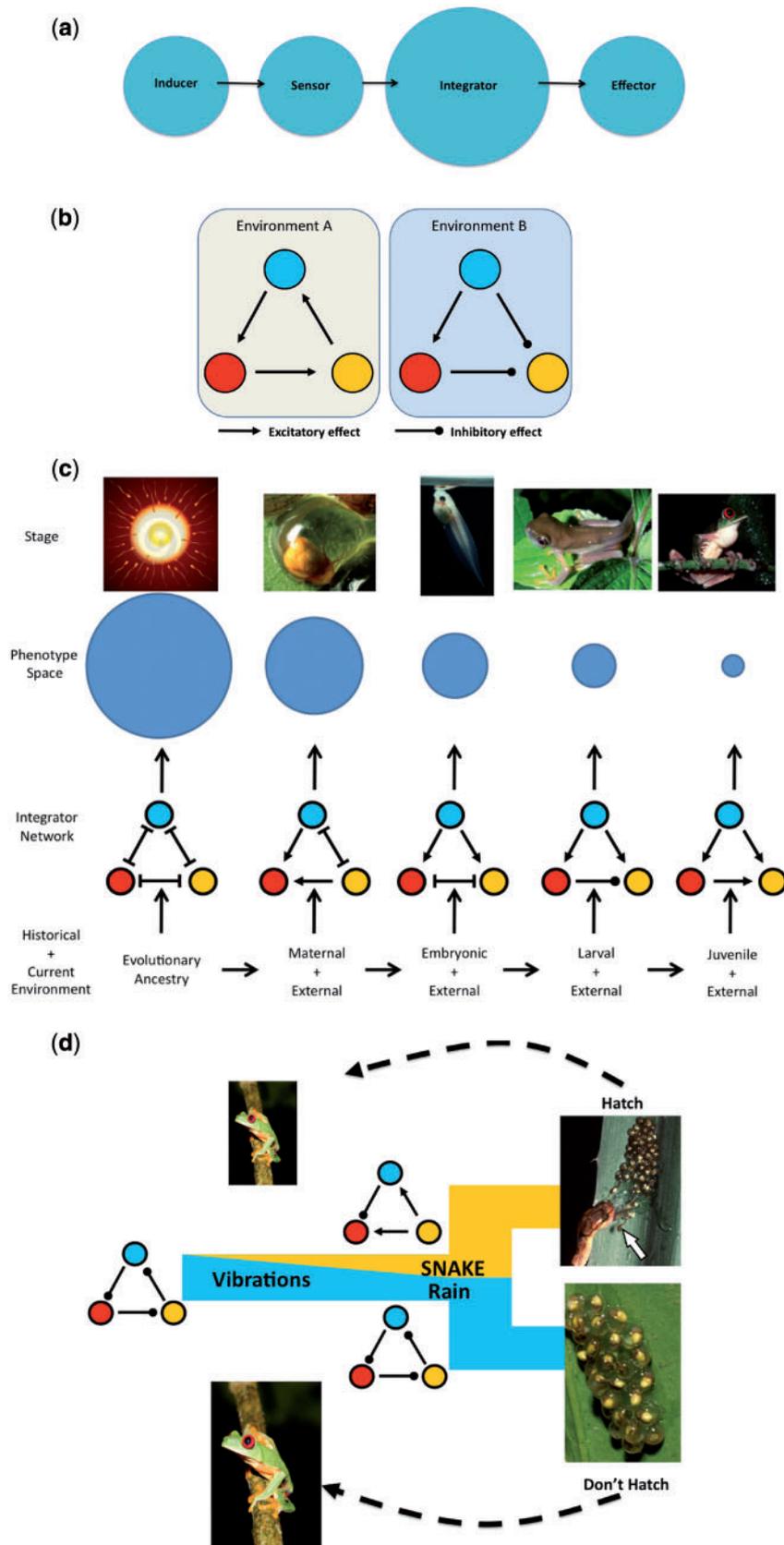


Fig. 1 Heuristic illustration of the characteristics and functions of integrators: (A) all physiological processes entail at least four elements with integrators serving as the potential link to other physiological systems. (B) Individual physiological systems interact as members of a network, here consisting of three integrators and how they might function in regulating phenotypes. Integrator networks

(continued)

allowing populations to persist under new conditions (Ghalambor et al. 2007).

Given the above effects of integrators, it is not surprising that they tend to be most impactful on the phenotype if engaged early in life (Monaghan 2008). Heightened sensitivity to integrators during early life may enable individuals (or their parents, or even their commensal/parasitic microbes) to adjust the phenotype of offspring/hosts to the environment likely to be experienced at reproductive maturity (Stetson et al. 1989; Gluckman et al. 2005). In fact, there is some evidence that experiences early in life are responsible for the behavioral and physiological syndromes (e.g., personalities, coping styles) commonly observed among individuals (Groothuis and Carere 2005; Bell 2007), and mounting evidence implicates integrators as influential to these phenotypes (Korte et al. 2005; Martin et al. 2005; Martins et al. 2007; Koolhaas 2008). Because integrators can have such strong effects early in life, it is also not surprising that many integrators are mediators of maternal effects. In vertebrates, testosterone (Schwabl 1993) and GCs (Schwabl 1993; Love et al. 2008) are passed from mothers to offspring and can impact the offsprings' phenotype profoundly. Exposure to embryonic testosterone in birds, in particular, increases aggression and dominance in adults and hence alters fitness, even though embryonic exposure does not affect the levels of testosterone circulating in adults (Partecke and Schwabl 2008). Recent research has demonstrated evolutionary implications of some integrator-driven maternal effects due to changes in the epi-genome of the offspring. For instance, the metastable A^{VY} epiallele of the *agouti* gene in mice can vary from hypo-methylated to hyper-methylated, and the degree of methylation determines the amount of expression of *agouti* with dramatic effects on individuals, including risk of diabetes and tumorigenesis. Most importantly from the integrator perspective, maternal diet supplemented with methyl donors (Waterland and Jirtle 2004) or embryonic exposure to a phytoestrogen, genistein,

increases methylation at the *agouti* locus, protecting against adult-onset obesity, among other effects (Dolinoy et al. 2006). Without question though, integrators and their network configurations are also important in adulthood as exemplified by sex reversals in fish (Pandian and Sheela 1995) and even subtler, "flexible" changes in phenotype (Piersma and Lindstrom 1997; Piersma and Drent 2003), such as intra-annual variation in reproductive readiness, immune functions, behavior, and morphology (Nelson et al. 2002).

Integrating integrators in theory and practice

There is still much to learn about integrators, but to determine whether the concept will be useful in predicting phenotypic variation in whole organisms, it will be first necessary to generate data in an appropriate model system. Due to their enduring, pleiotropic effects, however, integrators pose substantial logistical challenges for empirical and computational studies and integrator networks are even more challenging to study. Fortunately, recent advances in the study of evolving networks in computational sciences (i.e., vector-field editing) should guide us in collecting useful data, from which specific hypotheses can be generated.

The assumption of a one-to-one relationship between genes and phenotypes is largely untenable. Thus, successful computational approaches, as well as models describing the linkages between genes and phenotypes, have become loosely based around the concepts of *gene networks* (Pigliucci 2010). Recent studies have emphasized that the role of a particular gene for a phenotype is determined by the identity of the gene network within which it interacts and its position within that network (Chouard 2008; Stern and Orgogozo 2009), not just the gene itself. The phenotype is not translated directly from a genetic blueprint, but is an outcome of nonlinear systems created by interactions among multiple genes and their products (Stumpf et al.

Fig. 1 Continued

can be depicted graphically as a set of nodes linked with lines that depict the presence, strength, and directions of interactions among nodes. The activities of the nodes are influenced by the current state of the system, signals from other nodes in the network, and environmental information. Here, we illustrate two different network configurations for different environments. (C) Integrators convey information about both past and current environmental conditions. However, as an organism grows through ontogeny the degree of phenotypic plasticity that is possible (i.e., the phenotypic space that can be occupied) decreases. (D) An example of integrator effects in red-eyed tree-frogs (*Agalychnis callidryas*) illustrates how changes in the integrator network in response to external stimuli can lead to irreversible phenotypic decisions. Embryos detect a vibrational signal, and if the signal is indicative of a predatory snake then the yellow integrator (which might represent GCs) is up-regulated ultimately inducing hatching. In contrast, if the vibrations are indicative of rain then there may be no change in the integrator network and the embryos continue to develop without hatching. These two different responses can have both immediate (e.g., mortality without hatching in the presence of a snake) and long-term consequences for fitness (e.g., smaller size at metamorphosis for early hatchlings). The cat-eyed snake in Fig. 1D courtesy of Karen Warkentin, Boston University.

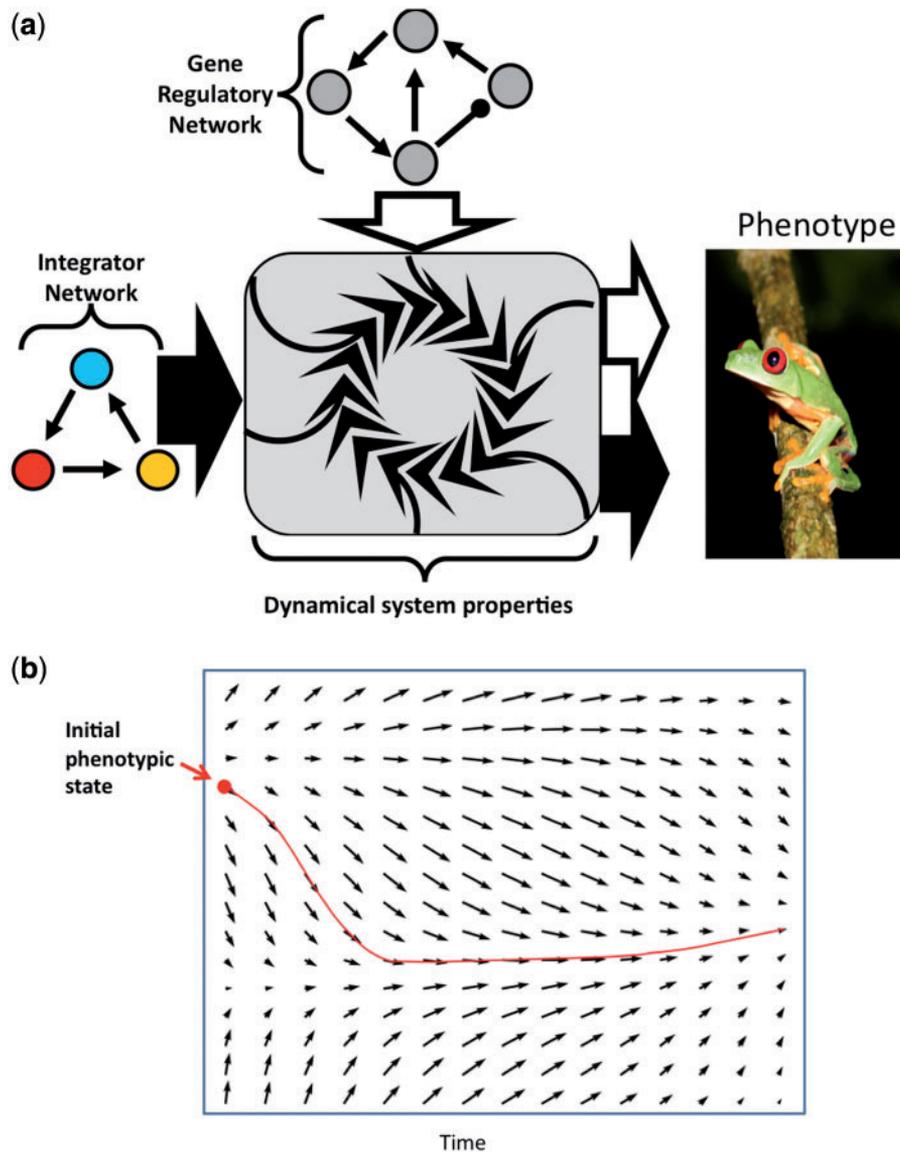


Fig. 2 Integrators can affect expression of the phenotype by manipulating the dynamic properties of the gene network (i.e., the sequence and identity of the gene modules expressed) without changing the topology of gene networks. **(A)** The interactions of a set of genes in a particular regulatory network create a phase space that results in a particular phenotypic outcome—path depicted by open arrows. However, the configuration of the integrator network can directly manipulate the dynamic properties of interactions among genes and change the phenotypic outcomes—path depicted by closed arrows. **(B)** Phenotypic trajectory across a 2D phase space, in which arrows (the vectors) describe the direction of change in phenotype as the state of the system changes in response to changes in the gene and integrator networks (modified from Steiner et al. 2009).

2008; Pigliucci 2010). The impacts of genes on phenotype are therefore most appropriately understood as elements of complex regulatory networks that change their expression dynamically in response to feedback from the environment (Wagner 2007; Newman 2010). Such networks are best depicted graphically as a set of nodes linked with lines that depict the presence, strength, and directions of interactions among genes (Fig. 1B). The activities of the nodes are influenced by the current state of the system, signals from other nodes in the network, and environmental information.

Integrator networks probably operate similarly to gene networks, but the tools necessary to test this possibility are only just emerging. One such tool, vector-field editing (VFE; Chen et al. 2007), provides a method for investigating whole-system variation focusing on the dynamic properties of the whole network and not the topology of the network (Steiner et al. 2009). VFE may also be amenable to understanding how integrator networks influence phenotypic variation (Fig. 2A). For instance, the interactions of a particular integrator network creates a phase space, which determines the phenotype, and

VFE allows direct manipulation and shaping of this space, which changes the system's dynamics and hence the phenotype in a causal manner (Steiner et al. 2009). VFE thus manipulates the direction of the path from genotype to phenotype and predicts the network configurations that could produce the final phenotypic state (Fig. 2B).

Understanding how genotypes are translated into phenotypes at the individual level is the main goal of the integrator hypothesis. However, population-level studies will also be important for determining the ecological and evolutionary ramifications of integrators. Such studies are best explored using life-table response experiments (LTREs), which provide a way to examine how changes in environmental conditions impact integrator networks and ultimately vital rates of populations (e.g., growth, reproduction, survival) (Caswell 1989). LTREs are useful because they permit the quantification of effects of integrators (alone and in concert) on different vital rates at different times. This disentanglement is important because the effects of integrators vary depending on the developmental stage and on prior activation of integrators. In addition, LTREs can reveal the influence of individual integrators, and the network as a whole, on rates of population growth (Caswell 1996), and they can also be used to examine how population demography changes as a result of fixed manipulations (e.g., stimulating/damping an integrator during a particular life stage), random perturbations, or along gradients. Thus, LTREs can be used to decompose stage-specific phenotypic impacts, carry-over effects, and latency of effects on demographic rates, which ultimately determine population dynamics and hence evolution. Regression-based LTREs should be particularly powerful for deriving multi-dimensional reaction norms and elucidating how plasticity is mediated by integrator networks. Implementation of response surface regression designs (Inouye 2001; McCoy and Bolker 2008) can be adapted to investigate how simultaneous changes in different integrators affect changes in other integrator systems, phenotypic variation generally, and population vital rates (e.g., survival, reproduction).

Even for model organisms, in which replication is not limiting, an outstanding and important question is what integrator traits should be measured in individuals. Clearly, organisms can alter integrator effects by changing the amount or affinity of receptors, circulating integrators themselves, and binding globulins, or gene-response elements (Zera et al. 2007). At the organismal level, we argue that measurement of the impacts of perturbation in one integrator on

other integrators in the network (e.g., the dose-sensitivity of immune or growth integrators to reproductive integrators among various windows of ontogeny, and their effects on the whole phenotype including fitness) will be most insightful. Whereas this approach may be unsatisfying at the physiological level, the practical difficulty of measuring a suite of components for all integrators will be overwhelming for almost any taxon. No matter what is measured though, practical limitations should not prevent the development of a viable theory of individual phenotypic variability.

Future prospects

Because the majority of physiological research comprises a single physiological system, our concept of integrator networks is presently based on indirect support. However, developing a theory of individual, organismal diversity will fill a major void in our understanding of the origins of phenotypic variation among individuals and the evolution of life generally. Below, we discuss a few implications of integrator networks, and how consideration of whole-organism physiology might change conservation practices as well as human health care.

Applied implications of integrators

Endocrine disrupting compounds (EDCs) mimic or block integrator effects (IPCS 2002), and include ubiquitous agricultural or industrial chemical pollutants such as organochlorines, plasticizers, and pharmaceuticals, as well as natural hormones such as phytoestrogens (McLachlan 2001). Although EDCs have the propensity to induce maladaptive, plastic phenotypic responses (Anway et al. 2005; Crews et al. 2007), EDC effects are often context-dependent. For example, one of the most commonly documented effects of exposure to EDC is feminization (IPCS 2002). However, the severity of the effects of exposure to EDC varies depending on when individuals are exposed (prenatally or postnatally) and the source of the EDC (e.g., synthetic or plant-derived) (McLachlan 2001). The context-dependence of these outcomes might arise if the integrator network buffers the effects of EDC depending upon its configuration. For example, European starlings (*Sturnus vulgaris*) that foraged on invertebrate prey contaminated with synthetic and natural estrogens developed longer and more complex songs, and enlarged HVC (a key brain area controlling the complexity of song) compared to control males (Markman et al. 2008). Males exposed to EDC, however, had reduced immune function, presumably driven by changes in

cytokines. These observations highlight the utility of the integrator network concept. For instance, EDCs may commonly have hump-shaped dose–response curves and phenotypic effects are context-dependent because other integrators buffer or enhance sensitivity to EDC (IPCS 2002).

In addition to understanding responses to environmental toxins in natural populations, the integrator concept should be salient for biomedicine. Although there is a vast literature describing the effects of single integrators in human disease, most integrator effects have not been studied as part of a coordinated network. A range of disorders are associated with altered regulation of GCs, including depression, obesity, metabolic and chronic fatigue syndromes, type-2 diabetes, and atherosclerosis. However, not all individuals with dysregulated GCs experience disease. We predict that disease arises not just through changes in GC signaling, but because of variation in the integrator network as a whole. Indeed, maternal psychosocial stress, leading to altered GCs, can decrease the length of gestation and weight at birth (Precht et al. 2007) and even contribute to coronary heart disease and type-2 diabetes in later life (Barker et al. 2002). Also, elevations of serum pro-inflammatory cytokine levels in pregnant females, which might occur due to stress or infection, can increase risk of allergy to the offspring later in life (Hamada et al. 2003). Incorporation of the integrator network concept should reinforce the growing understanding that genetic linkages with disease are unlikely to be simple (Feinberg 2007). Also, specific emphasis on integrator networks might enhance appreciation that other organisms (e.g., parasites and commensals) could exploit host integrators for their own benefit (Bailey et al. 2004). A growing list of examples demonstrates that some microbes can influence the development of the immune system (Litman et al. 2010) as well as mood and behavior (Forsythe et al. 2010) via integrators.

Conclusion

In some ways, the integrator concept is similar to previous endocrinological theories of phenotypic variation (Nijhout 1999; Jacobs and Wingfield 2000; Dufty et al. 2002; Hau 2007; Lessells 2008; McGlothlin and Ketterson 2008; Ricklefs and Wikelski 2002). What distinguishes the integrator concept though is that integrator networks, not single hormone effects, are emphasized. Phenotypic variation at the level of the whole-organism is explained, based on interactions among integrators as

well as on effects of single-integrators. A theory of whole-organism variation will not be easy to produce, but investigations of integrator networks should help fill the black box linking genotype to phenotype. Developing a more mechanistic understanding of individual phenotypic variation generally will further facilitate the necessary merging of developmental biology, ecology, and evolutionary biology (Pigliucci 2007; Nijhout et al. 2008; Pigliucci 2010). Effort should go to determining whether, and when, integrators provide adaptive benefits (Lynch 2007). However, the implications of integrators for nonadaptive variation (e.g., poor health [Nesse and Stearns 2008] or risk of extirpation due to integrator constraints [Martin et al. 2010]) is also critical. We do not propose that integrator networks encompass all there is to know about patterns and process in organismal diversity, but we do hope the ideas simulate efforts to achieve a complete Extended Synthesis in biology (Gottlieb 2002).

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Supplementary Data

Supplementary Data are available at *ICB* online.

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