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# Complexity Change During Physiological Development

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### Recognizing That Complexity Matters

At the supramolecular level, at least, most animals start out “relatively simply”—a haploid egg is fertilized by a haploid sperm, resulting in a single diploid cell. While the rich heritage of that animal’s lineage is contained within this cell’s genetic template, the fertilized cell itself is simple in structure. From these humble beginnings arise the enormously complex adult forms containing several hundreds of cells of numerous types in some metazoans (e.g., *C. elegans*) to the hundreds of trillions of cells in large endothermic vertebrates. More impressive than sheer proliferation of cell number during development, however, is the increase in organismal complexity that occurs as the fertilized cell repeatedly divides to form differentiated cell types that move on to form tissues, then organs, and finally organ systems. Indeed, the combined wonders and travails of this developmental journey would seem to be reflected in the recurring theme for book titles on the subject—*From Gene to Animal* (De Pomerai 1985), *From Egg to Embryo* (Slack 1991), and *From Conception to Birth* (Tsiaras and Werth 2002). As is evident from the proliferation of not only scholarly works, but also coffee table and even children’s literature, there is clear and longstanding interest in the developmental journey of animals—where it starts, where it finishes, and the steps in between—as well as an appreciation for the increases in complexity that occur along this journey.

Few would dispute that what we call “complexity” increases during development, but there have been few efforts aimed at a quantitative or even qualitative description of how physiological complexity changes during development. Animal physiologists have sometimes assumed that complexity increases in a generally linear fashion as development progresses. Thus, we often plan our experiments as if 1/3 of the way through development

animals are 1/3 as complex as when fully developed, 2/3 of the way through development are 2/3 as complex, and so on. For example, a fairly typical protocol for studying avian physiological development (and, admittedly, one that our own lab still regularly employs) involves arbitrarily dividing the incubation period into thirds (e.g., Dzialowski et al. 2002; Elmonoufy 2003; Chan and Burggren 2005), particularly in those studies searching for developmental critical windows. Yet, as will be described below, examples abound in which different physiological processes first appear and then begin to function at highly “nonsymmetric” intervals during development.

A corollary of the rather simple view that complexity increases linearly through development is that the more developmentally advanced the animal, the more physiologically complex it must be, culminating in sexual maturity and a presumed apex of complexity at this developmental benchmark. Yet, even a quick study of the natural history of many animals—both the typical models for developmental studies as well as the more diverse, less frequently studied species—reveals the sometimes enormous differences, if not absolute greater complexity, of intermediate developmental forms in both invertebrates and vertebrates (e.g., Hanken 1999; Hall and Wake 1999; Burggren 2005; Burggren and Warburton 2005; Hickman 1999). Consider, for example, the mobile nauplius larval forms compared to the sessile adults of barnacles, or the free-living larval ceratoid anglerfish that as adults degenerate into little more than a testes-bearing lobe attached to the female’s body wall. Even body mass, a trusted rule-of-thumb index of the progressive growth and complexity, can be deceiving. The paradoxical frog (*Pseudis paradoxus*) gains its name from the fact that it grows from a tiny egg of several hundred milligrams into the world’s largest tadpole (150 g) before rapidly falling down to a modest froglet of only a few grams (Burggren et al. 1992). It then grows again before reaching a maximum body mass of approximately 40–50 g as a mature adult. This paradoxical developmental change in body mass, representing a rather remarkable and rapid apoptosis in the middle of the life cycle, clearly flies in the face of our notions of progressive growth and complexity. Collectively, then, these few examples indicate that many intermediate embryonic, larval, and/or fetal forms can be argued as being *more* complex (and perhaps *far* more complex) than the terminal adult stage that supersedes them.

Clearly, a study of developmental biology—be it from a physiological, ecological, cellular, or any other perspective—begs the question “What is developmental complexity?” Rather like the concept of beauty, in which we (1) have difficulty in articulating a definition of beauty, (2) each feel we know beauty when we see it, and (3) often disagree with others as to what comprises beauty, similarly defining complexity during development is no easy task. Yet, it deserves elaboration, for “complexity” and how it changes is at the heart of developmental physiological studies.

The purpose of this chapter, then, is to:

- give examples of how complexity changes in nonintuitive ways during development;
- describe complexity and show how different types of biologists might view complexity differently;
- suggest how developmental physiologists might approach the issue of complexity changes during animal development; and
- consider insights into complexity from other sciences (e.g., computer science, mathematics, and materials science).

## How Do We Describe Complexity Change During Development?

### The Blind Men and the Elephant

An ancient Indian parable describes six blind men trying to describe an elephant, with each coming up with a vastly different description of the beast based on their differing experiences from touching only the trunk, the tail, the legs, and so on. The blind men and the elephant is not only a classic parable, but a classic example of what many scientists would call “sample bias.” Most biologists studying development have a notion of “complexity,” but their perspective, like that of the blind men, is restricted by their own training and background. An anatomist, for example, might view a developing animal as increasing in complexity if during the course of ontogeny it developed more structures (components). A physiologist would consider that same animal as increasing in complexity if it exhibited increasing numbers of processes, particularly regulated processes. An ethologist might view a developing animal as more complex as it began to show more intricate behaviors, at first intrinsic and then later extrinsically stimulated. A biochemist or cell biologist might view a growing list of enzymes and their isozymes isolated from the tissues of developing animals as an indication of greater complexity. As a final example, a molecular biologist might look at the proliferation of proteins as genes are expressed. Thus, each biologist, while certainly not blind but perhaps not seeing very far beyond their own discipline, views developing animals as increasing in complexity primarily if their own familiar metric increases in complexity.

Any one biologist could put together a fairly accurate picture of how complexity changes during development if all facets of a developing animal showed the same rate of appearance and subsequent increase in complexity; that is, if halfway through development an animal’s anatomy, physiology, biochemistry, behavior, and so on, were equally well developed. Importantly, this is not the case and, consequently, different types of biologists may end up interpolating and extrapolating quite differently back and forth through the developmental period they are studying. Consider as an example pulmonary system development in fetal mammals. The lung bud appears at day 26 after ovulation. By the end of the 16th week, all of the axial generations of the bronchial tree are in place. Differentiation of the alveoli occurs during the perinatal period (see Hodson 1977; DiFiore and Wilson 1996; Wiggleworth 1997; McMurtry 2002; Prodhan and Kinane 2002; Bourbon et al. 2005). The lungs also develop the full complement of cellular secretions (especially surfactants and related compounds) well in advance of birth or hatching (Daniels and Orgeig 2001; Blacker et al. 2004). Lungs are even “ventilated” with amniotic fluid through the fetal breathing movements developed by diaphragmatic and intercostal contractions (Cosmi et al. 2001). Yet, despite these apparent hallmarks of morphological and biochemical maturity of the pulmonary system, from a physiological perspective of gas exchange, the lungs have no function in respiration until that amazing “first breath” at the moment of birth. Thus, while the anatomist or biochemist might view the pulmonary system of a mammal as mostly complete immediately before birth, the physiologist might view it as still nonfunctional at that same point in development. Similar arguments could be made for anatomical maturity congruent with lingering physiological immaturity for the fetal liver, kidney, and so on.

It would be erroneous, however, to conclude that “anatomy matures early, physiology matures late.” Indeed, physiologists have long appreciated the very early functional role of the embryonic heart in gas exchange (though its early beating may be for angiogenesis rather than gas exchange: Burggren and Territo 1995; Pelster and Burggren 1996; Territo and Burggren 1998; Burggren 2004). The blood pressure and convective blood flow generated by the embryonic and then the fetal heart are absolutely critical to continuing anatomical development of other organs that may have no function until birth. Thus, mammalian cardiovascular physiologists would view cardiovascular function as changing, rather than growing, during fetal development, while a renal physiologist would not really get geared up for experimentation until birth, when osmoregulatory demands are suddenly thrust upon the neonatal kidneys.

### “The Whole is Greater than the Sum of the Parts”

Collectively, the examples cited above show that the pattern of change in complexity during development is likely to be viewed differently by different types of biologists, much like the six blind men each trying to describe the elephant in the Indian parable. The most accurate view of changes in complexity is then likely to emerge by an amalgamation or integration of diverse, and sometimes conflicting, views of complexity. To demonstrate this, let us examine the development of a system from anatomical, physiological, and integrated perspectives.

Consider as an example the respiratory development in an anuran amphibian such as a toad (figure 12.1A). While life histories vary greatly in anurans, let us follow a species in which the early aquatic larva (tadpole) starts out using its thin, gas-permeable skin for gas exchange with surrounding water. Perfused external gills quickly erupt from the body wall, but they are just as quickly supplanted by internal gill filaments born on the internal gill arches. These gills, ventilated by a stream of water, then form the bulk of aquatic  $O_2$  uptake, though the skin remains a major site for  $CO_2$  excretion (see Burggren and Just 1992 for review). As development continues, the paired lungs form and the larva begins trips to the water surface for air breathing (figure 12.1B). At this point prior to actual metamorphosis, the tadpole exhibits one of the most complex respiratory situations to be found in the vertebrates, as it is simultaneously using three quite different respiratory organs (skin, gills, lungs) to breathe with two very different respiratory media (water, air). Moreover,  $O_2$  and  $CO_2$  elimination are disproportionately skewed toward aerial and aquatic routes, respectively. As metamorphosis begins in earnest, the internal gills degenerate (an interesting story in apoptosis, there) and the lungs proliferate. Not surprisingly, then, subsequent metamorphosis to the toad’s adult morph in many ways leads to a considerable respiratory simplification. Complexity, measured either by number of components or number of processes, decreases as air breathing by the lungs assumes the main route for  $O_2$  consumption in the now fully terrestrial, air-breathing toad.

Now, let us examine this developmental change in complexity just described from three quite different perspectives (figure 12.2). Anatomically, respiratory complexity increases steadily in development as skin, then skin and gills, and then skin, gills, and lungs “come on line,” only to have the skin and gills eventually drop out. Physiologically, respiratory development shows a brief period of modest complexity increase just before metamorphosis when air breathing is added to the mix. However, if one looks at respiratory development from a combined or integrative perspective that considers the

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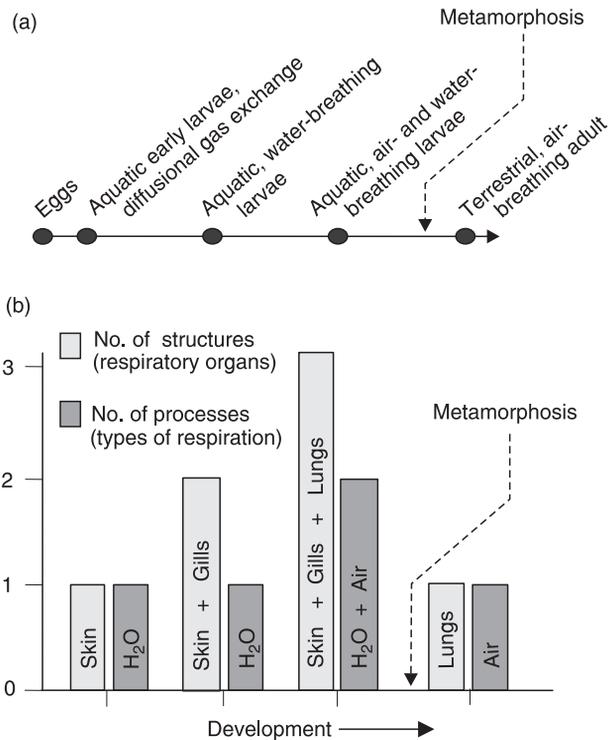


Figure 12.1 Changes in respiratory complexity during development in a toad with an aquatic larval form and a terrestrial adult. (A) The typical life cycle of a toad involves an intermediate larval stage during which there occurs a transition from aquatic to aerial respiration. The larva eventually develops lungs to supplement its gills and skin, and then eventually loses its gills at metamorphosis and depends almost entirely on the lungs for  $O_2$  uptake. (B) Changes in respiratory complexity based on either the number of respiratory structures (skin, gills, lungs) or the number of respiratory processes (skin breathing, gill breathing) during development of a terrestrial toad. Note that the change in respiratory structures and the change in respiratory processes both peak in late larval life, when the animal is concurrently using gills and skin to exchange gas with water, and lungs to breathe air.

number of possible structures *and* the number of possible interactions—and expresses complexity as the product—then complexity surges at the time of metamorphosis. Of course, a direct interaction between “lungs” and “water” is unlikely (or at least is a once-in-a-lifetime event), but important secondary interactions can link processes and components. For example, a decrease in water  $P_{O_2}$  (potentially rendering cutaneous and branchial respiration less effective) can reflexly stimulate breathing and pulmonary gas exchange with air in amphibian larvae (see Burggren and Just 1992), with there also being a complex temporal component of the interaction between lung and gill ventilation depending on the interbreath interval (West and Burggren 1983).

As is evident from this example of amphibian respiratory development, the most accurate view of complexity and how it changes during development occurs when complexity is evaluated in the context of:

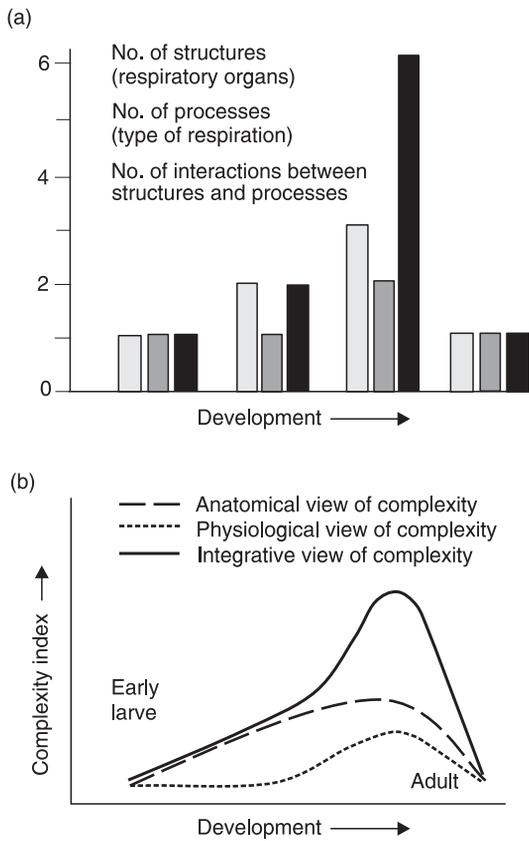


Figure 12.2 An interaction-based view of changes in respiratory complexity during development in an anuran amphibian. (A) The solid bars show the possible interactions, that is, the *product* of the number of structures and number of processes, at each stage. This product reveals the potential number of interactions between all respiratory components, and shows that this measure of complexity greatly amplifies the apparent complexity in late larval stages. (B) Description of the changes in respiratory complexity during amphibian metamorphosis varies greatly depending on whether one takes an anatomical view, a physiological view, or an integrative view (the product of anatomy  $\times$  physiology).

- the number of components involved;
- the number of processes involved;
- the number of interactions between components and processes; and
- the time frame over which these components and processes change.

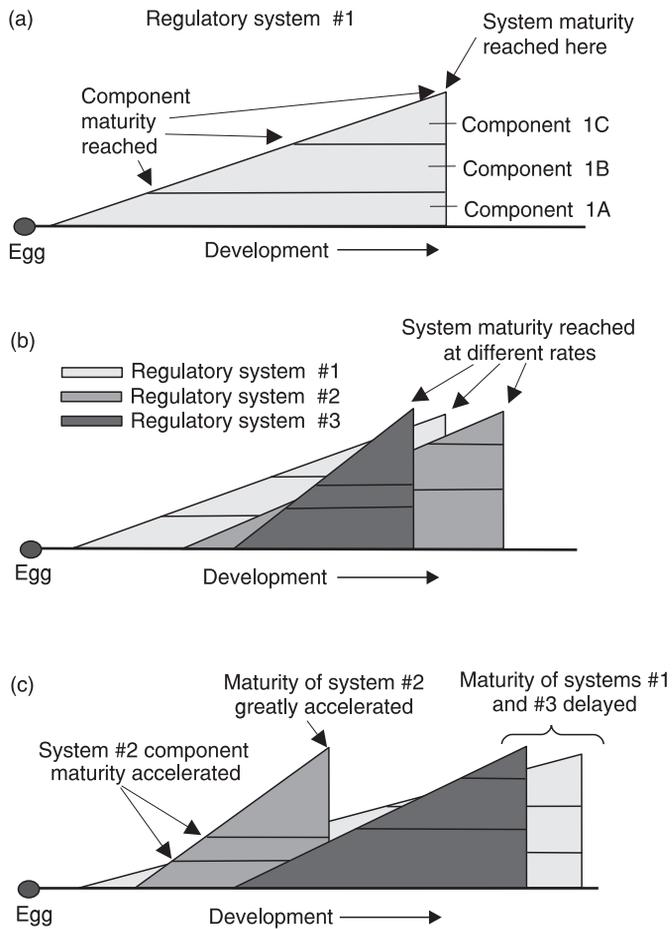
Later in this chapter we shall explore how not all interactions are two-way, which leads to systems that are absolutely less complex, but also less intuitively evident. In the meantime, however, I would argue that comparative physiologists, if not comparative anatomists, have potentially underestimated how complexity changes during development by not considering the potential interactions of parts (anatomy) and processes (physiology).

### Environmentally Induced Developmental Change—"Heterokairy"

To this point, we have considered the ontogeny of physiological complexity as if genetically fixed. Yet, consider that the interplay of multiple components of multiple regulatory systems is also likely to be modified during development by environment or maternal effects (Spicer and Burggren 2003). To explore this notion, let us first consider the ontogeny of a single, simple regulatory system composed of three components (figure 12.3A). This system will not become functional until the full development of all three components, each of which is likely to develop at different times and different rates. An example might be a baroreceptor reflex, consisting of afferent neurons, efferent neurons, and the baroreceptor itself. Now, to layer on additional complexity, consider *three* cardiovascular regulatory systems within a developing animal, each composed of three components (figure 12.3B). These systems, like the components that form them, may all become functional at different rates, and at different times in development. Thus, regulatory system 1 could be the baroreflex already described, regulatory system 2 might be a slightly more slowly developing chemoreflex (with its own afferents, efferents, and an O<sub>2</sub>- or CO<sub>2</sub>-sensitive receptor), and regulatory system 3 might be an osmoreflex (likewise consisting of efferents, afferents, and an osmoreceptor), which is the slowest of the three to develop.

Apart from the obvious explosive increase in number of components and processes to keep track of, how does this relate to environmental alteration in complexity? Consider that evidence is starting to accumulate from experiments on a variety of both invertebrate and vertebrate embryos and larvae that shows that environmental perturbations and experimental hormonal manipulations can alter the *relative* rates of first appearance and subsequent development of physiological regulatory systems; that is, physiological developmental programs appear quite flexible. Thus, perturbations in environmental hydration and oxygenation lead to changes in the regulation of variables such as embryonic lung surfactant levels, blood osmolality, blood oxygen affinity, blood volume, blood pressure, cardiac output, and O<sub>2</sub> transport during development (see Warburton et al. 1995; Morritt and Spicer 1996; Crossley and Burggren 1997; Spicer and El-Gamal 1999; Blacker et al. 2004), while treatment of larval freshwater salmonids with cortisol, growth hormone, and insulin-like growth factor can accelerate the onset of seawater tolerance and associated physiological changes in their gills (McCormick et al. 1991; McCormick 1994). Importantly, these induced changes appear to be the equivalent of altering the time and rate of onset of each of these three regulatory systems, as shown schematically in figure 12.3C. Thus, the onset of regulation by one system may be "brought forward" just as that of another might be "sent back" in developmental time. These changes may, or may not, lead to real changes in the fitness of the developing animal (though this could be determined experimentally).

The relative change in timing of the onset and development of physiological regulatory systems (or indeed, of any aspect of development) *within a single animal's ontogeny* has been termed "heterokairy" (Greek; *hetero* = different; *kairois* = at the right time) by Spicer and Burggren (2003) to clearly distinguish such change from heterochrony (changes in development over *evolutionary* time). Clearly, the presence of heterokairy in developing systems increases complexity by adding the additional dimension of the effects of acclimation/acclimatization on the genetically dictated developmental program.



**Figure 12.3** The ontogeny of multicomponent, multisystem regulation in a hypothetical developing animal. (A) A regulatory system composed of three different components, each first appearing and then maturing at different times in development. Only when all three components are complete is the system truly functional. (B) Three different regulatory systems, each composed of three different components, may develop at different times, and at different rates. (C) The pattern of development of various regulatory systems as well as the components forming each system may be altered by environmental perturbation, potentially accelerating the development of one system and its components, perhaps at the expense of the development of others. This pattern of change within a single animal's development is referred to as *heterokairy* to clearly distinguish it from heterochrony, that is, changes in the rate of timing of development over evolutionary time. (After Spicer and Burggren 2003.)

### Insights into Complexity from Other Scientific Disciplines

Biologists of all kinds working on developmental changes in complexity are becoming increasingly interested in the perspectives of other scientific disciplines studying the “development” (i.e., growth) of nonbiological complex systems. Particularly interesting

and timely advances have been made in mathematics, physics, computer science, and material sciences (for entry into literature, see Burggren and Monticino 2005).

### Mathematical, Axiomatic Approaches

Mathematicians have a rich history of studying complexity (one of the mainstays of the renowned Santa Fe Institute, as one example) and are beginning to interact with developmental biologists (but as yet few physiologists; see Burggren and Monticino 2005). While the true quantitative modeling of complexity change during physiological development will require a concerted collaboration, we can gain insights from considering just one such melding of mathematics and biology. Nehaniv and Rhodes (2000) have described five axioms for recognition of complexity in biological systems, which should be readily extendable to *developing* biological systems. Some of these axioms are truisms, but taken as a whole, the five axioms they advocate provide an enlightening framework for considering biological complexity. It is beyond the scope of this chapter to elaborate fully on these axioms and assign numerical “complexity scores,” as Nehaniv and Rhodes (2000) have. However, let us briefly consider each axiom, its description, and its interpretation with respect to physiological complexity in developing systems.<sup>1</sup>

#### **Initial Condition Axiom**

**Description:** “Certain trivial systems have complexity zero.”

**Interpretation:** Developing physiological systems have minimal complexity (at the macro level). While this axiom is quite obvious, it does set up the view that complexity can only increase—but in what pattern and at what rate?

#### **Constructability Axiom**

**Description:** “A biological system is the sum of low-complexity, interacting components.”

**Interpretation:** Like the Initial Condition Axiom, this axiom is rather self-evident, but does highlight the point that one cannot talk about the complexity of an organism based on the complexity of a single system that one happens to be studying.

#### **Part-Whole or Covering Axiom**

**Description:** “A single component contributing to complexity cannot be more complex than the system itself.”

**Interpretation:** If one discovers that, for example, branchial respiration in a larval amphibian is a complex process, and if there are additional nonbranchial respiratory organs, then in fact overall respiration must be even more complex than you have imagined by just looking at gill function.

#### **Noninteraction Axiom**

**Description:** “Complexity only increases if the combined components actually interact.”

<sup>1</sup> These axioms are presented out of the order in which Nehaniv and Rhodes (2000) developed them, but in increasing order in terms of my perceived usefulness to developmental physiologists.

**Interpretation:** This is a particularly intriguing axiom, because it speaks to the issue of the potential disparate views of a physiologist and an anatomist viewing a developing animal. In developing animals, many components are present but as yet non functional, and certainly have not begun interacting with their neighboring tissues and organs. So, for example, an embryo, fetus, or larva may have kidneys, adrenal glands, a heart, baroreceptors, and so on—that is, be anatomically complex—but may not yet be capable of short- or long-term blood pressure regulation because these system have not yet begun to interact in physiologically complex ways.

#### **Bounded Emergence Axiom**

**Description:** “Interaction between components increases complexity, but one-way interaction sets bounds on the possible increase.”

**Interpretation:** The simple mathematical laws of probabilities that would define a total number of possible interactions do not automatically apply when considering the interactions of developing structures and processes. So, for example, a developing animal might have two processes (A, B), but while process A affects process B, the reverse may not be true. Thus, there is only one rather two possible interactions between these components. Consider as an example a developing endocrine organ, which might be mature enough to influence a target organ, but the complete feedback loop controlling that endocrine organ may not yet be mature enough to be fully functional.

Collectively, these and other such axiomatic approaches should prove helpful in organizing our intuitive, commonsense impressions into a more rigorous, structured framework that is more likely to generate testable hypotheses. There are many other fruitful approaches to the mathematical modeling of complexity, rooted to various degrees in the real world, that could profitably be brought to bear on studies of changing complexity during development, physiological or otherwise.

### Computer Science and Self-Organizing Systems

Computer scientists have been considering complexity almost since the inception of their discipline, and of course are solving real-world situations that grow and develop and become more complex (e.g., expanding the data transfers associated with the ATM network for a growing national bank). Of particular interest to developmental physiologists should be advances in so-called “self-organizing systems” being promulgated by computer scientists studying artificial intelligence and data retrieval. Such systems are composed of many small, individual components randomly inserted into an environment. Importantly, such environments lack a “central authority,” a key concept in the world of self-organizing systems. Economists, for example, would define a central authority as a Chief Executive Officer, while political scientists would view a government as the central authority. Extending this concept to physiology, the neuroendocrine complex might be viewed as the central authority.

Though lacking a “central authority,” the components of self-organizing systems, when given a few surprisingly simple intrinsically programmed rules, can generate complexity from apparent randomness. They can come together to collaborate in carrying out common functions, and can maintain self-organized criticality (a dynamic but stable configuration). Computer-generated birds called “boids” (imagine a computer screen of small, randomly moving symbols) can fly in an eerily lifelike “flock” when programmed with three simple rules: (1) don’t crowd, (2) match your neighbor’s actions,

and (3) move to the middle (Reynolds 1987). Building upon this conceptual base, more recent collaborations between computer scientists and neurobiologists are discovering how similarly simple guidelines dictate the actual learning processes in self-organizing systems (e.g., Principe et al. 2002; Seiffert and Jain 2002; Tani 2003; Uthmann and Dauscher 2005).

Before we consider the relevance of self-organizing systems lacking a central authority, let us consider “real-world” self-assembling systems.

### Materials Science and Self-Assembling Systems

Self-organizing/self-assembling systems have been taken from the virtual world to the real world by materials scientists working in nanotechnological applications. Impressed with the wealth of examples in biology for self-assembly, such as protein synthesis or neural network assembly (Aggeli et al. 2001; Camazine et al. 2001; Seiffert and Jain 2002), nanotechnologists are developing processes by which components are mixed together and poured as a thin film onto a surface such as a silicon wafer. The mixtures, sometimes guided in their assembly by incorporation into the mixture of biological structures like pieces of nucleic acids, then assemble themselves into SAMs (self-assembled monolayers) which can function as electromechanical transducers, data storage devices, and so on (Nolfi and Floreano 2000; Davis and Higson 2005). SAMs and other self-organizing systems represent the most thermodynamically stable of all possible organizations, and consequently tend to have two important attributes:

- SAMs are relatively defect-free, which is vitally important in the electronics industry; and
- SAMs are capable of self-repair.

Michael Crichton’s (2002) *Prey* provides an entertaining yet chilling fictional account of how self-organizing and self-assembling systems can operate without a central authority—indeed, can challenge the central authority.

### Central Authorities and SAMs: What Does it Mean for Physiological Development?

Can theorems, hypotheses, and experimental outcomes in mathematics, computer science, and materials science be used to understand how dividing cells might organize themselves into physiological systems, bereft of a “central authority”? More specifically, to what extent can we view the developing organism as a “self-organizing, self-assembling system,” and if we allow ourselves this approach, what insights can we glean about the development of physiological systems and their regulation? To provide a possible answer to these questions, consider the growth and development of a hypothetical animal.

Traditionally, we view the growth of the embryo as grossly divided into two phases (figure 12.4). In the first phase, the animal is without functional nervous and endocrine systems. Since it lacks a “central authority,” we presume that coordinated, regulated response to environmental challenge cannot occur. As an example, early in development the larva of *Xenopus laevis* shows no cardiac response to hypo- or hypertension, because the nervous system has not matured sufficiently to enable baroreflexes (Warburton and Fritsche 2000). Similarly, avian embryos with otherwise highly functional circulations

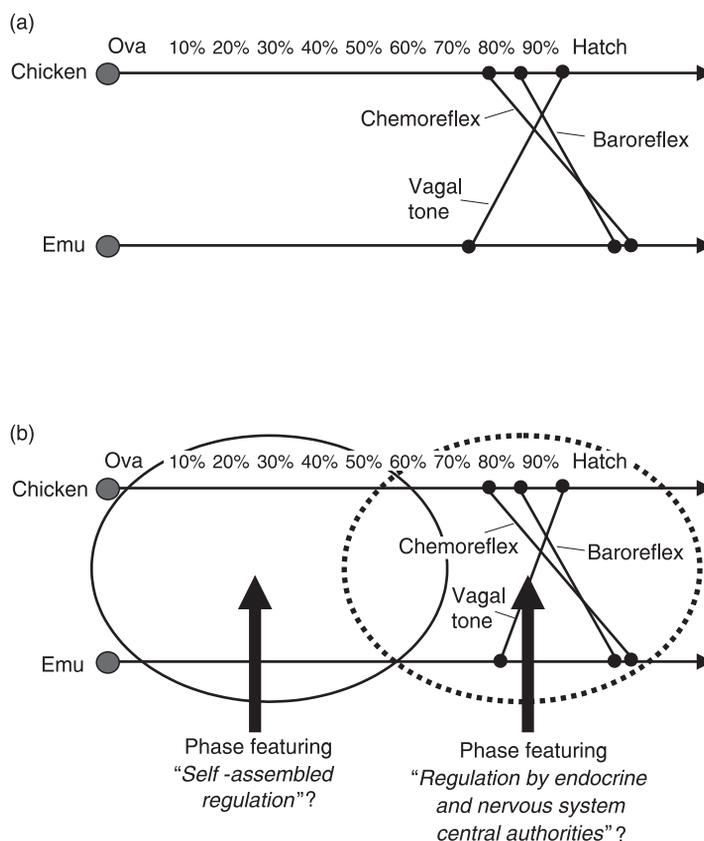


Figure 12.4 Two proposed phases of physiological regulation revealed in time lines for reflex development in avian embryos. (A) In the chicken and emu, central nervous system regulation of the heart, evident in chemoreflexes, baroreflexes, and vagal tone, appears in the last third of development (after Burggren and Crossley 2002). (B) Development in these avian embryos may be generally characterized into an early phase of “self-assembled regulation” prior to nervous and endocrine system development, and a later phase characterized by regulation by these late-developing physiological central authorities.

fail to respond to hypoxia because of the lack of fully functional neural/hormonal reflexes (Burggren and Crossley 2002).

Continuing with the traditional view, embryos in the second phase of physiological development eventually develop physiological central authorities (brain, endocrine organs) and the “workers” that will respond to their commands (effector tissues and organs). This presumably allows for the first time coordinated physiological response to changes in the internal milieu. Indeed, the study of the development of physiological regulation is replete with examples of regulatory systems becoming functional at discrete points in ontogeny as new regulatory components develop, mature, and interconnect with each other.

If, however, we consider that the artificial systems capable of self-assembly and self-organization being developed by materials and computer scientists are far simpler than

those of biological systems, is it not logical to allow for the far more complex and sophisticated biological systems to be capable of these demonstrated characteristics of self-organization? That is, might the individual components of an early developing embryo actually be able to generate coordinated responses to environmental perturbation? In truth, we have not examined the physiology of developing "phase 1" embryos in sufficient depth to determine whether they are simply very tolerant of environmental insult, or whether they are actually capable of mounting "self-organized" responses in the absence of intact neural/hormonal regulatory components.

### Thermodynamics and Development?

Finally, developmental physiologists (and developmental biologists) might consider actively exploiting the simplicity of the protosystems developed by computer scientists and nanotechnologists, for it may be in these simple systems that we are able to understand whether there is a thermodynamically based minimum set of requirements by which the components of animals assemble themselves. The most accurate self-assembling/self-organizing systems are created using thermodynamically based rather than operator-controlled manufacturing processes (Allara et al. 1992; Ulman 1996; Saksena and Woodcock 2005). So, for example, rather than creating a set of environmental conditions by an operator-controlled "on-off" process (that is, by turning on the manufacturing process and then turning it off when the desired product is achieved), the most accurate and precise systems are created by thermodynamically controlling the assembly. All of the energy for self-assembly typically comes from either the chemical reactions themselves or from the thermal activation of the reaction temperature. For example, self-assembly of alkane-thiol monolayers on gold films is driven by the formation of the Au-thiol bond. The more Au binding sites occupied, the better is the monolayer quality. Once all available Au binding sites are occupied, alkane-thiol monolayer assembly stops, there being no thermodynamic reason for it to continue (B. Gnade, pers. comm.). Such thermodynamically regulated manufacturing processes are far more precise than a process in which an operator turns on and off a manufacturing process at specified times.

Extending the notion of thermodynamic limitations and control to developing animals, perhaps animals develop the way they do because it is thermodynamically most advantageous. How such a thermodynamic view of development would map onto our current knowledge of organizers, morphagens, and homeoboxes has yet to be determined. Yet, from Russian biologists come a series of provocative books and articles that introduce the potentially complex interplay between ontogeny and development; see, for example, Aleksandr Zotin and his colleagues (Lamprecht and Zotin 1988; Zotin 1972, 1990; Zotin and Lamprecht 1982) and Gladyshev (1996). While discussing these papers is beyond the scope of this chapter, consider the rich ore ripe for future mining evident in this single quote from Gladyshev's (1996) highly quantitative modeling paper:

The chemical composition of the phase of supramolecular structures of the biological system slowly changes at times comparable with the duration of adaptive processes and ontogenesis, as well as during phylogenesis and at long-term stages of biological evolution as a whole. With the biological tissue senescence, the supramolecular structures become more thermodynamically stable (the supramolecular structures themselves, rather than the chemical substances that form these structures). (Gladyshev 1996, p. 390)

Clearly, theory is way out ahead of data with respect to thermodynamic control over development, but increased collaboration of developmental biologists with “thermodynamicists” can only accelerate our understanding of developmental physiology.

## Conclusions and Future Directions

As physiologists interested in development, we face numerous challenges as we deal with the “why” and “how” of complexity changes during development. Specifically, we would be well advised to:

1. Acknowledge that “complexity” has many facets, and defies a simple definition.
2. Provide whenever possible a rigorous definition of “complexity” in developing animals, because with complexity clearly defined we can then design experiments to determine the interactions of the components, processes, and interactions leading to that complexity.
3. Recognize that the rate at which complexity increases during development both waxes and wanes in regulated systems, and that not all systems show changes in complexity at the same time, or the same rate. Related to this, we also must recognize that the rate of change in physiological complexity may be modified by environmental conditions during embryonic, larval, or fetal development.
4. Learn from other scientific disciplines that have been more successful at revealing basic tenets governing self-assembly and self-organization in the absence of central authorities. Examples might include working on models or actual physicochemical systems that are intrinsically simpler than living systems. Principles emerging from such studies may reveal as yet unappreciated mechanisms by which cells differentiate and assemble into complex anatomical systems producing sophisticated physiological processes.

Future studies, then, would be profitably directed to understanding the role that previously unappreciated (or underappreciated) phenomena such as self-assembly play in development, and how they contribute to the changing complexity of physiological regulation during the development process. In this regard, it will be important to design experiments to test whether early embryos are mounting a coordinated response to environmental challenge, or rather are composed of cells that individually are highly tolerant to such insult. Also worthy of further study is the role of thermodynamics in physiological development. To what extent do animals develop the way they do because it is simply most energetically favorable to do so? Also largely untapped is the study of the evolution of physiological complexity during development. While this touches upon phenomena such as heterochrony and *heterokairy*, there have been few studies that have set out to catalog ontogenetic changes in physiological complexity and how such complexity evolves. Finally, experiments need to be designed to look more critically at the interactions *between* organ systems—their anatomical components and the physiological process they support. We cannot fully understand developmental changes in regulatory complexity without looking at the broader context in which organ systems begin to function.

When we have learned how to define, recognize, alter, and model developmental complexity, then, quite ironically, we shall have greatly simplified our pathway to understanding animal ontogeny.

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