Organisms
Journal of Biological Sciences

Vol. 2, 2 (December 2018)
ISSN: 2532-5876
DOI: 10.13133/2532-5876_4.7

ORIGINAL ARTICLE

Atavism: Accessing Ancient Attractors Hidden in the Epigenetic Landscape

Sibel Yildirim[a] *and Sui Huang*[b]

[a] *Pediatric Dentistry, Selcuk University*

[b] *Institute for Systems Biology, Seattle WA, USA*

Corresponding author: Sui Huang **sui.huang@systemsbiology.org**

Abstract

Atavism, the reappearance of an ancestral trait in an individual organism of a species that lacks that trait, such as hind-legs in whales or teeth in chicken, is considered an accident of development. But far from a destructive error, it manifests a stunningly complex, organized structure indicative of a creative-constructive process. The contradiction between rarity of an accident and rule-obeying nature of a common, yet sophisticated anatomical form remains a challenge for the common explanation that atavism results from genetic mutations that reactivate evolutionarily silenced developmental genes in the genome. Here we propose that an atavistic trait can be understood as the re-accessing of a remnant ancient attractor in the high-dimensional dynamics of the gene regulatory network (GRN) not meant to be occupied. Attractors are stable configurations of gene expression patterns, represented by "potential wells" in a quasi-potential landscape, which in turn is the mathematical equivalent to Waddington's epigenetic landscape. This article reviews the formal concept of the attractor landscape and how its topography changes following mutations that rewire the GRN, thus allowing evolution to remodel the landscape. Such changes of the landscape channel developmental trajectories to new attractors while leaving old attractors behind as hard-to-access side-valleys, some of which would encode the atavistic traits. Then, atavism represents the accidental entry into these latent ancient attractors due to either new mutations or developmental plasticity enforced by environmental perturbations. Implications for the interpretation of specific cases of atavism are discussed.

Keywords: attractor states; gene regulatory networks; quasi-potential landscape; Waddington; complex systems; developmental plasticity

Citation: Yildirim, S, Huang, S, 2018, "Atavism: Accessing Ancient Attractors Hidden in the Epigenetic Landscape", *Organisms. Journal of Biological Sciences*, vol. 2, no. 2, pp. 59-80. DOI: 10.13133/2532-5876_4.7

1. Introduction

Biologists have long been struck by the constructive nature of developmental anomalies that organisms are prone to produce. When something goes wrong in embryonic development, the results are not simply the absence of an organ. Often they are manifest as characteristic, complex, sometimes monstrous formations: double-headed amphibians, additional pairs of wings or eyes, legs at the place of antenna, humans with tails - the list is almost infinite. Even without knowledge of the specific underlying molecular aberrations that would offer a mechanistic explanation of the monstrosities, we intuitively accept that creative deformations reflect the enormously complex but rule-governed nature of development. For, such behavior is consistent with our daily experience with complicated systems: Random rotation of a kaleidoscope will delight us with unseen yet familiar, beautifully symmetric patterns – even if we fail to produce a desired one. A small defect in the wiring of an aging TV set does not necessarily result in a dark screen but often expresses itself in characteristic, interestingly distorted pattern of the screen picture –caricatures of normal function, often reminiscent of older, less functional models, such as loss of colors. We intuit that these patterns arise because of some internal constraints: A complex system of multi-layered, hierarchical control structures pre-vents a random non-fatal

perturbation from pushing the system into the structure-less chaos, and instead produces characteristic symptoms often consisting of a combination of familiar elements.

One particular type of creative malformation in organismal development is *atavisms* – from the Latin word '*atavus*', meaning a great-grandfather's grandfather and, thus, more generally, an ancestor. 'Atavism' denotes the reappearance of an ancestral type in the anatomy of an organism. In atavisms tissues and organs appear to revert to express characteristics found in a distant evolutionary ancestor (Hall, 1984). The stunning creative-constructive effect of a developmental accident, if we consider atavism one, is the production of a structure that epitomizes an organized ancestral structure.

The most salient examples of atavism have indeed a flair of a caricature, such as humans with tail-like formations of their sacral spine (Bar-Maor et al., 1980) or teeth in chicken, supernumerary nipples in humans or hind-legs in whales (Louchart and Viriot, 2011, Galli-Tsinopoulou and Stergidou, 2014) (Box 1). But more subtle cases of reversion to ancestral traits at the biochemical and cellular level have also been described, sometimes in experimental settings, such as in bile acid conjugation patterns (Brueton et al., 1978). Cancer has been considered as a cellular atavism in which cellular traits of early protozoa-metazoa reemerge (Vincent, 2012, Davies and Lineweaver, 2011).

In the early literature, the apparent violation of the principle of eliminating "less fit" ancestral traits by natural selection had been perceived as an embarrassment to evolutionary biologists, as illustrated by synonyms for atavisms: evolutionary throwbacks, backward step or setback of evolution (Gould, 1994, Hall, 1995, Ashley-Montagu, 1938, Sandeman, 1898, L, 1898). Specifically, the dismay most probably stemmed from "Dollo's law". This central principle of evolution states that an organism is unable to return, even partially, to a previous stage already realized in the ranks of its ancestors (Dollo, 1893). According to Hall (Hall, 2003), "Dollo did not deny reversibility entirely, only that complex structures could not be recreated. Dollo's law can now be viewed against knowledge of the genetic and development bases of the formation of structures." Although this law implies that the degradation of genetic information is fast enough that developmental pathways and associated organismal structures will rapidly become nonfunctional, thus freeing them from selection (Marshall et al., 1994), the reappearance of a lost trait challenges our understanding of deeper mechanisms of evolution. Atavisms also challenge the gradualist's account of evolutionary transitions (Gould, 1977), according to which one group of organisms evolves from another through a graded series of intermediate forms.

Box 1. EXAMPLES OF ATAVISM

Atavism is the appearance of a lost trait that was present in a distant evolutionary ancestor but not observed in the immediate ancestors (or the offspring) of the organism carrying the atavistic trait. Examples:

- Hind legs on whales or snakes
- Hind fins on dolphins
- Extra toes on horses (polydactyly, the second and forth digit), as in archaic horse
- Residual thigh muscles in perching birds and true sparrows
- The reappearance of wings in several lineages of stick insects
- Re-emergence of sexual reproduction in the flowering plant Hieracium pilosella and the Crotoniidae family of mites.
- Teeth in chickens
- Hyoid muscles in domestic dogs
- Reversional fibular bones in birds
- Polydactyly in guinea pigs and possibly salamanders
- Pelvises and hind-leg buds in snakes
- Reevolution of shell coiling in Gastropoda after 10 million years
- Restoring to the aberrant fly Drosophila's ancestral complemet of four
- Examples in human
- Vestigial tail, "coccygeal process" and "caudal appendage"
- Congenital generalized hypertrichosis in humans ("werewolf syndrome")
- Extra nipples
- Large teeth, resembling those of other primates
- Snake heart
- Reappearance of ancestral lifestyles (behavioral traits)

The importance and intellectual implications of the very concept of atavism are far-reaching because of the peculiarity of the atavistic structures compared to other malformations. While the sheer diversity of capricious

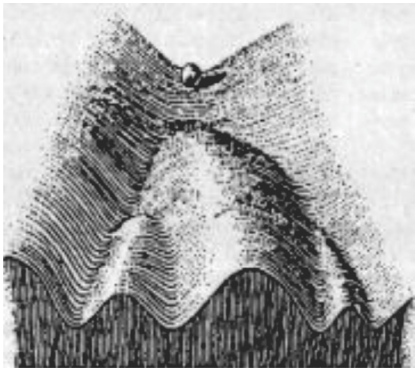
manifestations of errors in the intricate machinery of development, as manifest in tera-togenic birth defects, is intuitively plausible in view of its complexity ("so many ways that something can go wrong"), the reversion to a structure of high complexity once expressed by evolutionary ancestors as seen in atavism may still test our intuition as well as conceptualization of evolution as a sculptor of natural forms towards increasingly complex functionality. If a defect in a color TV set produces a black-and-white picture, this reversion to a feature of early models can readily be explained by the loss-of-function of the mechanisms that produce color, such that a more primitive functionality is exposed –a picture devoid of color. By contrast, atavistic tissues not only represent ancient, more "primitive" forms, but they also are not inherently simpler. Teeth, tails and legs are complex structures that cannot be explained by the dysfunction of a more recent functionality. Nor can atavism simply be explained as a rare "gain-of-function" mutation, one of those rare fitness-increasing mutations that produce "hopeful monsters" as envisioned by evolutionary biologist Richard B. Goldschmidt. He was one of the first to suggest that gradual evolution by random variation and natural selection may not suffice to explain evolu-tionary dynamics (Goldschmidt, 1940, Goldschmidt, 1980). But atavistic developmental anomalies, while rare, are still too frequent, too organized and too often replicates of ancestral forms to be considered simply the consequence of the rare and unique "macro-mutations" that Goldschmidt envisioned played a role in the generation of new species.

To more formally explain these elementary features of atavism one must consider the following essential principles: (i) retention of ancient forms by some type of memory, (ii) their efficient suppression in the wild-type individuals and (iii) some sort of saltatory, yet constrained defect – that is, a simple molecular defect, possibly in just one pathway, with large-scale phenotypic effect. This article will provide a conceptual framework based on the dynamics of networks of interacting elements (genes, cells) that will naturally account for these three central principles.

There is no lack of contemporary attempts to explain atavism in molecular and cellular terms. However, most existing explanations employ the very same conceptual framework as those used to explain complex phenomena in development and evolution that has come to dominate modern biology: the reduction of observations to linear, deterministic molecular causation (Noble, 2008, Huang and Wikswo, 2006, Strohman, 1994, Tauber and Sarkar, 1992). There, complexity is achieved by additive multi-factorial causation that can be linearly parsed into independent contributing factors – a epistemological habit most prominently epitomized by GWAS (Genome-wide Association Studies) in which one seeks genome-sequence variants that are statistically associated with a phenotype (disease) of interest (Cirulli and Goldstein, 2010).

At the core of such reductionist view is a prevailing climate of thought that tacitly assumes a rigid one-to-one, or at least a simple, mapping between genotype and genotype. Such thinking has elevated proximate causation by a molecular pathway to the universal explanatory principle of biology. This view has been criticized at length and found to be unsuited to deal with organismal complexity (Huang, 2012a, Pigliucci, 2010, Noble, 2006, Soto and Sonnenschein, 2005, Longo et al., 2015). But with the arrival of systems biology that embraces systematic and exhaustive molecular dissection yet seeks to integrate the parts to an holistic entity (Huang and Wikswo, 2006) and to understand "emergent" structures, molecular pathways are not simply the biochemical embodiment of linear chains of causation but part of complex regulatory networks that govern development. Now, time is ripe to revisit atavism in this new light.

In this article we offer an interpretation of the causes of atavism from the perspective of non-linear dynamics of gene regulatory networks (GRN), an old idea that in the past years has matured both at the theoretical and experimental front. Notably, the theory of the integrated (global) dynamics of GRN has provided a firm formal foundation to Conrad Waddington's metaphoric concept of an "epigenetic landscape" (Figure 1) that he used to explain natural developmental trajectories and the predestined course of cell phenotype (Huang, 2012a). Thus, Waddington's useful conceptual metaphor of a landscape is now linked to molecules and mathematics, and is formally called the *quasi-potential landscape* of a dynamical system, namely the GRN. The formal definition stifles tendencies of hand-waving in the use of an intuitive and convenient metaphor to explain apparently irreducible phenomena. At the center of our thesis presented herein in a permissively simplified (qualitative) manner, is the idea that an atavistic feature manifests the accidental or perturbation-induced entry of a developing system into "abandoned attractors", so-to-speak locked-away side-valleys hidden in the epigenetic landscape whose topography is molded during evolution by rewiring of the GRN that mathematically defines the shape of the landscape.



Fiure 1. Waddington's "epigenetic landscape" (Waddington, 1957).

In the more mature 1957 version (Figure 1), the marble on the top represents a cell and its geographic position the *cell state* – or in modern parlance, the state ***S*** of the gene regulatory network (GRN) that is in every cell. The GRN governs the production of a gene expression profile that defines the cell state and implements a particular cell phenotype. In an extended, higher-level scale view the marble can be seen as representing an entire tissue (instead of a cell), with its position then indicating the *tissue state* (instead of a cell state). A tissue state is then controlled by the cell-cell interaction network (instead of the GRN), and defines the tissue state through the composition of the cell types (and their states) in the tissue and their location.

The elevation of the landscape maps into the modern, formal concept of the quasi-potential U that expresses the "relative stability of states". The lowest point in the valley (lowest potential) would then represent a stable state – an attractor state. The topography of the landscape is shaped by evolution through rewiring of the GRN as genes mutate and represents the entire developmental repertoire of the GRN – hence, of the cell or tissues. During development the marbles roles down to the attractor states (bottom of valleys), which represent the mature differentiated cells (or tissues) states. In Waddington's metaphor, gravity pushes the marble to seek the lowest points on the landscape; it is a force that epitomizes the driving force of the GRN as a dynamical system, and emanates from the regulatory actions that genes exert upon other genes in the GRN. These regulatory interactions collectively give rise to the attractor states in state space and causes cells to move to them, as explained in this article. The path of the marble (cell) on the landscape to the adult attractor states are the trajectories of GRN dynamics and correspond to what Waddington called 'chreods'. They have been carved by evolution of the landscape, such as to avoid getting stuck in "side valleys" which may represent atavistic phenotypes (see Figure 2, 3).

2. Atavism: defining features and related phenomena

Let us briefly review the very phenomenon of atavism. In "The Variation of Animals and Plants under Domestication", Darwin discussed the subject of atavism (Darwin, 1868):

> The cases of reversion may be divided into two main classes, which, however, in some instances, blend into each other; namely, first, those occurring in a variety or race which has not been crossed, but has lost by variation some character that it formerly possessed, and which afterwards reappears. The second class includes all cases in which a distinguishable individual, sub-variety, race, or species, has at some former period been crossed with a distinct form, and a character derived from this cross, after having disappeared during one or several generations, suddenly reappears. A third class, differing only in the manner of reproduction, might be formed to include all cases of reversion effected by means of buds, and therefore independent of true or seminal generation. Perhaps even a fourth class might be instituted, to include reversions by segments in the same individual flower or fruit, and in different parts of the body in the same individual animal, as it grows old. But the two first main classes will be sufficient for our purpose.

Later Hall suggested three basic settings for atavism (Hall, 1984):

1. As spontaneously occurring phenomenon in natural populations (limbs of vertebrates, extra toes in modern horses, extra nipples and coccygeal projection (tail) in humans, atavistic muscles in birds and, miscellaneous other atavisms);
2. In selective breeding of laboratory animals (extra toes in guinea pigs, atavistic dew claws in dogs, hypertrichosis
3. As experimental induced structure (reestablishment of ancestral patterns in the hind limb of embryonic chicks, enamel from avian ectoderm, balancers, teeth and gills in amphibians, bristle pattern in Drosophila, atavistic growth of teeth in birds or reptilian features of skeletal system in birds).

In order for a trait to qualify as an atavism, it should '*re-*'appear as a '*lost*' trait: one that was present in a distant evolutionary ancestor but not observed in the immediate ancestors of the organism carrying the atavistic

trait (Tomic and Meyer-Rochow, 2011). The high degree of phenotypic resemblance to the trait in the ancestor of distant relatives along with the appearance as a fully developed character in adult life stages and the scarce occurrence in populations distinguish atavistic trait from character homology (Hall, 1995). By contrast, a *vestige* is a version of a character found in ancestors that occurs in adult stages but is incompletely developed and interpreted as a consequence of loss of function and hence, is not maintained by positive selection pressure. On the other hand, a *rudiment* occurs in the embryonic stage as a partially formed feature. Finally, if the trait occurs across entire phyla rather than infrequently within a population, then it is referred to as '*phylogenetic character reversal*' (Tomic and Meyer-Rochow, 2011). However, in *phyletic* or *taxic* atavisms all members of a species share the character (Hall, 1995).

More recently Zanni and Opitz took attention to reconsideration of certain reasonable criteria, including (Zanni and Opitz, 2013):

- "Homology of structure of the postulated atavism to that of ancestral fossils or collateral species with plausible soft tissue reconstructions taking into account relationships of parts, obvious sites of origin and insertion of muscles, vascular channels, etc.
- Most parsimonious, plausible phylogenetic assumptions.
- Evident rudimentary or vestigial anatomical state in prior generations or in morphogenesis of a given organism.
- Developmental instability in prior generations, that is, some closely related species facultatively with or without the trait.
- Genetic identity or phylogenomic similarity inferred in ancestors and corroborated in more or less closely related species."

3. The current molecular view of developmental mechanisms of atavisms: "epigenetics"

Attempts to explain atavisms in terms of the physical or molecular mechanism(s), in the same sense as we seek to explain the development of normal traits have been scarce. Most probably, this is because atavisms is surrounded by the aura of anecdotes or because atavistic traits have not been accepted as phenomena, unlike vestiges, that could teach us something about evolution (Gould, 1994). The frequency of naturally occurring atavisms has been underestimated although specific instances of ancestral recurrences abound (Tomic and Meyer-Rochow, 2011). Hall suggests that "*the developmental mechanisms responsible for the reappearance of ancestral structures is often responsible for prolonged growth of an element that is normally rudimentary because it fails to maintain an early growth rate*" (Hall, 1995).

After several of his breeding experiments on reversion in both pigeons and poultry, Darwin concluded reversion did occur. His Pangenesis hypothesis elaborated to explain the mechanism of reversion along with many other genetic phenomena (Li and Liu, 2014). According to Pangenesis, reversion was due to the long-dormant ancestral gemmules (minute molecules in cells, as Darwin called them before the notion of 'genes' was established in the early 20th century) becoming active after the transmission of many generations (Liu, 2005).

While selective breeding of laboratory animals (as in the case of atavistic polydactyly of in guinea pigs) can reveal a genetic foundation of atavism (Hall, 1984), the sudden appearance of as complex a program as the naturally occurring rudimentary hind limbs in some whale species (Hall, 1984), *polythelia* (supernumerary nipples) (Galli-Tsinopoulou and Stergidou, 2014), or the coccygeal projection (tail) in humans (Dubrow et al., 1988), and in experimentally induced atavistic growth of teeth in birds (Louchart and Viriot, 2011), are indeed counterintuitive and not readily accommodated by the simple genotype-phenotype relationship. Zanni and Opitz state (Zanni and Opitz, 2014):

> The Darwinian concepts of pangenesis and telegony, and the "dormant gene" hypothesis of Zuckerkandl and Pauling have met with skepticism in the past because of lack of understanding of their theoretical basis. But, with the advances of genomics, epigenomics, and the uncovering of new forms of transmission of genetic information, we cannot exclude that the underlying molecular mechanisms will contribute to a causal elucidation of the origin of atavisms.

According to the Encyclopedia of Genetics, Genomics, Proteomics and Informatics (Redei, 2008), having real basis in the genetic material, such as hypertrichosis in humans, encoded in chromosome Xq24-q27.1 (Figuera et al., 1995), atavistic traits can have a straightforward Mendelian scheme of inheritance. "*If a developmental program shift can activate an altered form of ancient genetic sequences, atavistic changes may be expressed.*" Likewise, Benham et al. proposed (Benham et al., 1995):

> The reappearance of an atavistic trait requires a mutation that induces expression, probably by reactivating a dormant set of genetic instructions or by causing some regulatory mechanism to revert

to an earlier mode of control. This mutation then is propagated to descendants by Mendelian inheritance. If this trait confers an advantage, either for survival or for mating, it can become fixed in the descendant population.

3.1 Two types of epigenetics

Therefore, as so often in modern biology when a simple genetic causation fails, an immediate intellectual reflex is to steer discussions of causes of atavism toward the domain "epigenetic mechanism". This shifts uncertainty and inaccuracy of thought to another level, for the term 'epigenetic' itself suffers from having multiple meanings– a discrepancy rarely explicitly articulated, albeit its inappropriate usage by modern molecular biologists has been criticized (Ptashne, 2007, Oliveira Pisco, 2016). In the broadest sense, 'epigenetics' is generally used to describe any phenomena that require an alternative explanation to *genetic* alterations ('epi' = beyond, 'above') without further mechanistic specification. But more specifically, in common modern usage 'epigenetics' can indicate two distinct phenomena – depending on the background of the author:

First, 'epigenetics' in mainstream contemporary (molecular) biology most commonly refers to chromatin modification by a set of covalent modifications of DNA or/and of histone proteins that influence gene expression through modulation of physical accessibility to genomic DNA by the conformation of chromatin (see 3.2. below). In this meaning epigenetics is thought to convey enduring states of gene activity to each gene locus, in the way as genetic mutations do.

Second, the historically older use of 'epigenetics' refers to the differentiation and stabilization of phenotypes by interactions within intracellular molecular networks that regulate cell behavior and by interactions between cells and tissue and the physico-chemical surrounding (section 3.3.). The fact that the dynamics of interactions between the components of a system can encode a stable system state is typically explained by self-reinforcing regulatory feedback loops. But below we offer a more formal explanation. The networks of interactions are said to sustain "epigenetic states"; they collectively embody the ability of a single genome to produce a multiplicity of stable, enduring phenotypes. This second meaning contains Waddington's intention when he coined the term 'epigenetics' (Waddington, 2012, Huang, 2012a, Oliveira Pisco, 2016).

3.2 Epigenetic chromatin modifications: continuation of the reductionist view

The covalent DNA and histone modifications, also referred to as "epigenetic marks", offer a com-fortable rescue of the reductionist view of genetic determinism, for they replace genetic mutations with "epimutations" and thereby allow for changes in the activity of gene loci that are subjected to environmental influences and can confer an enduring memory of such influences. The idea that placing a molecular epigenetic mark on top of the genomic sequence without altering can encode an environmentally induced, lasting phenotype, while at the same time preserving the option for reversion to the original state later on, and thus could explain atavism, is attractive. Epigenetic modifications would allow for the regulatory suppression of entire gene programs over generations, and would also account for non-genetic (environmental) factors in their reactivation.

But this explanation suffers from the same insufficiency as the general invoking of epigenetic marks in development and disease to account for the multiplicity of alternative, stable phenotypes that one genome can produce. Molecular epigenetics is characteristic of the reductionist way of thinking that seeks molecular proximate causation and preservation of the dominance of the genome as source of all explanation, while accommodating phenomena that defy the rigid 1:1 genotype-phenotype mapping. But epigenetic modification of individual gene loci can neither explain the complex phenotypes that result from the collective, coordinated action of genes nor explain their dynamics that drives a directional change of a complex phenotype (Huang, 2012b). Enzymes that "write" the epimutations on the chromatin, such as DNA methyltransferases and histone (de)methylases (Kouzarides, 2007), do not recognize specific DNA sequences and thus can by themselves not coordinate the action of genes (Huang, 2012a). Moreover, each covalent modification is opposed by enzymes catalyzing the reverse reaction, thus the memory property of "epimutations" must stem from something else (Trojer and Reinberg, 2006, Kubicek and Jenuwein, 2004). If epigenetic modifications control epigenetic "programs" – then who controls the controller? Coordination requires regulatory networks with feedback loops - cyclic causation.

3.3 The need for gene coordination to explain complex traits

Epigenetic modifications offer a proximate explanation concerning the regulation of the expression of one gene locus at a time (activation/repression) by describing one part step of the underlying molecular mechanism. They do not take into consideration the coordinated expression of multiple genes that are involved in the development of an atavistic traits, a limb, for example. Thus, while one could envisage that the retention in suppressed form of complete sets of morphogenetic instructions in the genome for millions of years may be mediated by epigenetic modification, it is obvious that the coordinated regulation of the specific set of loci will require orchestration by a regulatory network. Thus, epigenetic marks are not the *primum movens* of a change of gene expression but the follower. The chromatin modifying enzymes must be recruited by transcription factors, which provide the "locus-awareness" that is required for coordinating the activity of a specific set gene loci through their ability to discriminate the DNA sequences of the regulatory elements flanking the genes. Hence transcription factor are the critical elements for the orchestration of biologically coherent "gene expression programs". In addition, while "epi-mutations" (Peltomaki, 2012) have been used as excuse when genetic mutations cannot be invoked - either because of the high frequency of reversion observed or the failure to find the anticipated mutations in genomic sequences - there is a fundamental difference: epigenetic marks are set in a regulated fashion, often following a cell's response to external signal, and not randomly, as are genetic mutations. Hence, epimutations are not the randomly generated substrate of selection but the result of directed environmental instruction.

The reductionist perspective relies on chromatin modifications to understand novel phenotypes but in doing so fails in the same way for explaining atavism as they fail to explain the organized cell phenotype diversification in development (Huang, 2012a). On the other hand, the complexity of atavistic traits suggest that a regulatory dysfunction in a master control system within a regulatory network must play a role in the same way as in the case of the familiar homeotic mutations, such as the Drosophila bithorax mutant (which can be considered a form of atavism) (Lewis, 1978) or the artificial ectopic limbs induced by ectopic overexpression of master regulators (Schneuwly et al., 1987).

3.4 Beyond epigenetic modifications towards a network-based explanation of atavism

The above discussion on the simplistic attribution of atavism to epigenetic control of individual gene loci to account for de-repression of ancient "genetic programs" makes it clear that coordination of gene expression across large sets of genes is central. Thus, the most plausible explanation offered so far (Tomic and Meyer-Rochow, 2011) is that atavism follows the reactivation of a preexisting "genetic *program*" by somatic or germ-line mutations that affects the gene regulatory network (GRN). Invoking a regulatory network and "genetic programs" would help explain the development of complex traits the way homeotic mutations produce grossly abnormal morphologies. But this concept still is merely an *ad hoc* qualitative explanation and raises the question of what does the metaphor of a "genetic program" stand for in the first place? A satisfactory account for the specific constraints underlying the development of ancestral traits not used for generations, yet typical and recurring, thus robust, is needed.

Therefore, a satisfying explanation of the infrequent but highly distinct and robust proclivity for the reappearance of a complex ancestral trait needs to address: (1) the coexistence of *plasticity and constraint* in phenotype regulation that permits small (likely random) genetic or non-genetic changes to trigger a coherent ("constructive"), complex phenotype changes; (2) the specific nature of the phenotype change towards an ancient phenotype; and (3) the ability of the genome and GRN to shutting off a trait, preserve some memory of it and reactivate it many generations (and speciation events) later. Meeting this challenge will entail departing from the habit of proximate molecular explanations and embracing fundamental, more abstract principles and integrative approaches, while not losing sight of molecular interactions, which after all embody the material basis of all biological phenomena.

An explanation shall also meet the following more profound requirement. Any satisfying theory of a natural process must address its "directionality" or "spontaneity", that is, the "inevitability" in the breaking of the time-symmetry from within, without invoking an obvious "upstream" or external cause (Prigogine, 1997), and also avoid the notion of an *a priori* purpose. (In biology, the idea of purpose often comes in the guise of natural selection, and is epitomized by the reduction of a function to a gene *for* that function). To analyze the principles behind the directed phenotype changes that underlie development of an atavistic structure we will

introduce two major sets of ideas that will be applied onto each other and serve as guiding concepts. (1) The concept of "atavistic attractors" and how they emerge from gene regulatory network dynamics – this will give the vague term of a "program" a more specific meaning; (2) The concept of joint action of chance and necessity in driving evolutionary change, an idea that is outside the familiar scheme of mutation/selection of individual genetic mutations (Huang, 2012b). Here, necessity reflects intrinsic constraints in a system of interacting components whose collective action embodies the departure from linear causation and the 1:1 genotype-phenotype mapping.

4. From gene regulatory networks to attractors to Waddington's epigenetic landscape of development

To explain our thesis of atavism we will take two steps into a more abstract realm: First (4.1.), we will present the concepts of attractors, the formal basis for coherent programs in the genome; second (4.2.), we will describe how the multi-attractor dynamics of the genome can be seen as a process that takes place on the epigenetic landscape. The latter can serve as a formal substrate of evolutionary change.

4.1 Cell types as attractors on the epigenetic landscape

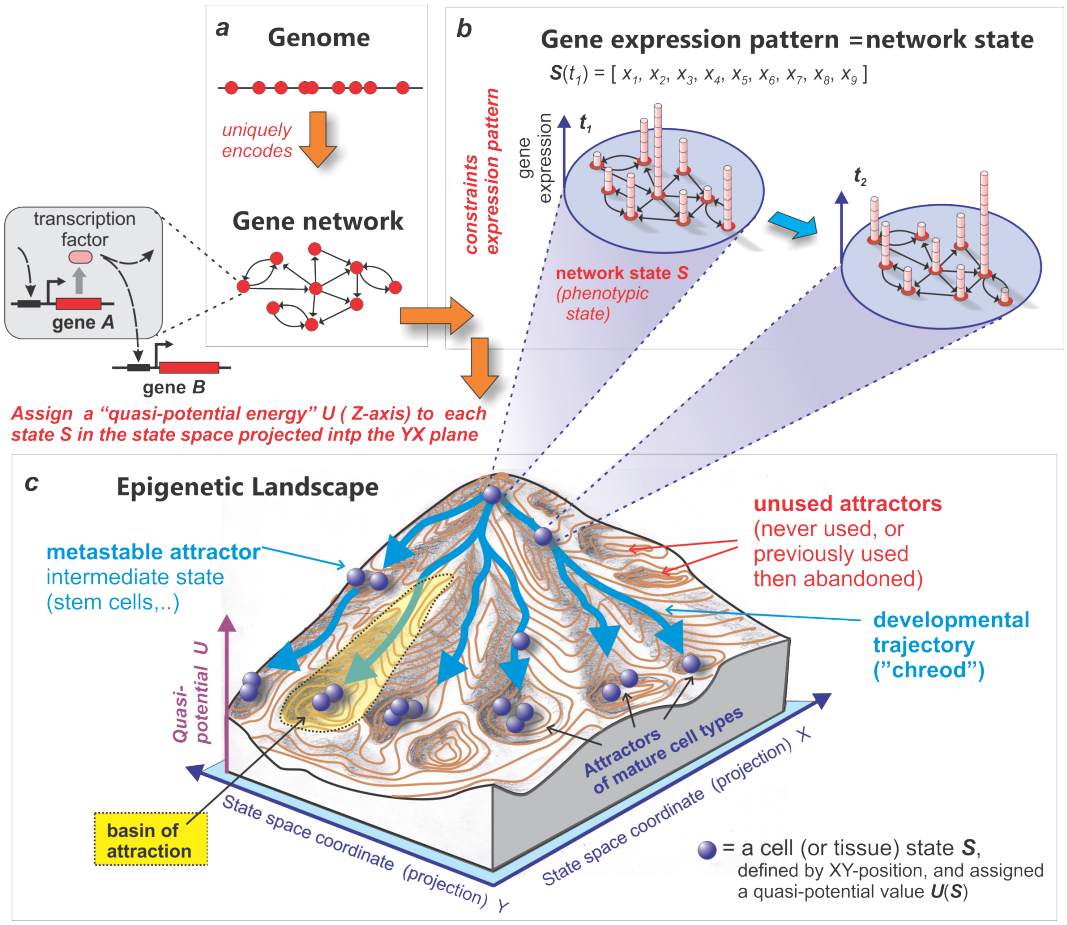
In the first step we establish the molecular and mathematical basis of Waddington's epigenetic landscape, which serves as a tool for a formal de-scription of the relationship between genome and a complex phenotype. We will explain qualitatively using a cartoonish, permissively simplified picture, how the collective behavior of all the regulatory genes that form a network of defined regulatory interactions can be displayed as a landscape. These interactions are encoded in the genome through the specificity of molecular interactions of transcription factors (promoter binding, protein-protein binding, etc.), which in their entirety constitute the "wiring diagram" of the GRN. It is in this sense that the GRNis "hard-wired" in the genome. However, this does not at all imply that the phenotype is "fixed" by the genome. In contrary, this distinction is important for understanding developmental plasticity, as we will see later.

The regulatory interactions orchestrate the expression of all the gene loci of the genome to produce distinct *gene expression profiles S*, each of which is a configuration of the expression level values (e.g. gene ON or OFF) across all the gene loci of the genome. Each gene expression profile, *S* thus also represents a state of the GRN and also, at some approximation a cell state - or cell phenotype.

A central principle is that the regulatory interactions embodied by the GRN *constrain* the gene expression changes at individual gene loci, thereby restricting the possible configurations of gene expression *S*, such that the majority of theoretically possible gene expression profiles are not stable and hence, biologically not realized. Figure 2 illustrates these constraints on gene expression of *S* configurations. Without entering into mathematical formalism, it suffices here to point to the consequence of such mutual dependence of gene expression among the genes: The reason why most configurations *S* are not stable is because they violate regulatory interactions of the GRN. For instance, if gene *A* (unconditionally) suppresses expression of gene *B*, then all combinations in which both genes *A* and *B* are highly expressed would be unstable. However, a few configurations of gene expression *S* comply with regulatory rules of the GRN and are thus stable. These distinct stable gene expression profiles are called "attractors" of the GRN.

Attractor states can formally be shown to correspond to the bottom of some sort of "potential wells" in a ***quasi-potential landscape***, which can be regarded as a mathematical representation of Waddington's epigenetic landscape. In this historical metaphoric picture, the valleys and hills guide the marble – representing a cell– to the lowest points at the bottom of the valleys or "lowest quasi-potential states" (Huang, 2012a, Zhou et al., 2012) (Figure 2). Waddington's landscape concept thus captures though the gravitational pull towards the lowest elevation the driving force behind the collective change of expression of all the genes, orchestrated by the regulatory interactions between the genes. More formally, each point (position) on the landscape represents a gene expression profile or a network state *S*. Thus, an attractor state is a particular network state.

The picture of a potential well, familiar from classical physics or chemistry (where unlike here, we have "true" energy potentials and not "quasi" potentials), illustrates the relative *stability* of attractor states to each other. Roughly, the lower a valley, the more "stable" is the attractor state. Relative stability refers to the comparison of two attractors and expresses the "relative ease" (probability) for moving from one attractor to the other given some random fluctuations in the gene expression state (Zhou et al., 2012). Thus, the landscape



Fiure 2. Basic concepts from gene regulatory network to the epigenetic landscape. A genome (a) encodes the information for the gene regulatory network (GRN) or "gene network" (b) through the DNA-binding domains in transcription factors and the cis-regulatory elements of all genes (inset) to which the transcription factors bund. A black arrow represents a regulatory interaction. The GRN orchestrates the gene expression pattern (the large purple circle) mandating that some patterns are more stable than others because the co-expression of individual genes has to obey the rules imposed by the GRN, resulting in gene expression patterns (network states) S having distinct "quasi-potentials". Gene expression patterns that satisfy all regulatory rules of the GRN are stable and called *attractor states* (or *attractors*) and encode biologically relevant gene expression patterns ("programs"), such those that define a cell types. The notion of a quasi-potential values allows us to assign to every network state *S* an "elevation" representing the quasi-potential *U* (***c***). For visualization, if the high-dimensional space of all network states (the "state space") is compressed and projected onto a 2-dimensional plane (XY-plane, blue) onto which one can for each state *S* (defined by its XY-position) map an elevation –the quasi-potential *U* of that state *S*; this results in a contiguous landscape since similar states (which are neighbors in the XY plane) typically have similar quasi-potential values (elevations). The lowest point in a valley (potential well) is an attractor states and all points in a valley that "drain" into that attractor state form its basin of attraction (yellow). Development is the distribution of cells, moving along the chreods (blue arrow), into the attractor states that encode functional cell or tissue phenotypic states. For mathematical reasons, the complex GRN produces many more attractors (valleys) on the landscape than are utilized by the organism to represent all cell or tissue states. Some of these unused attractors had been used in the evolutionary past – they are remnants of evolution as the landscape grew and new chreods were carved to bypass them (see Figure 3).

captures, along with the subliminal notion of gravity, the notion of the direction of spontaneous cell state change. Deep attractors, like those representing normal differentiated cell types, are easy to enter, but hard to escape – they are relatively stable compared to their neighboring attractor states,

Technically, stability thus implies resilience to (small) perturbations that affect the expression level of any number of genes in a given state *S*, such as molecular noise that causes fluctuations in gene expression levels (Eldar and Elowitz, 2010, Raj and van Oudenaarden, 2008). Upon such a perturbation, the system (GRN) falls back to the lowest point in the potential well, and thus reconstitutes the gene expression profile that represents the attractor state. Thus, if a gene expression profile represents an attractor state, then the associated gene expression profile determines the levels of expression of thousands of genes characteristic for a stable cell state that is self-sustaining. This means that the system can "correct itself" when the expression of the genes fluctuate due to noise or is perturbed, and their expression level deviates from that which defines the attractor state. The attractor thus "attracts" similar (neighboring) states that are not stable. In other words,

cells with unstable gene expression profiles will "role down" to the bottom of a well, or "attracted" to the attractor state, and in doing so adopt the stable gene expression profile defined by the respective attractor. This property of stability with respect to essentially all genes in the genome is utilized by nature for "molecular homeostasis" – the maintenance of the appropriate expression level of all genes across the genome to keep a stable gene expression profile, hence cell phenotype.

It is in this sense that a distinct cell phenotype, such as a nominal *cell type* has been explained by an attractor state – a concept that has its roots in the ideas of M. Delbruck, Mono and Jacob and S. Kauffman concerning the differentiation (Huang, 2011). Since one genome, that is, one GRN, can generate thousands of stable attractor states, a phenomenon called "***multistability***", we have now a formal conceptual framework that explains how one genome can generate a multiplicity of stable, enduring phenotypes that can resist minor perturbations. Once in an attractor state, it is difficult but not impossible for a cell to leave it – a process that requires *coordinated* change of expression of multiple genes – which is achieved by natural signals (via the signal transduction apparatus) or artificial GRN manipulation as in cell fate reprogramming. The process of development is then essentially the regulated displacement on the epigenetic landscape of the multiplying cells towards occupying the appropriate attractor states in appropriate proportions to generate the cell type patterns of tissues.

The existence of many attractor states in the dynamical behavior of a network, or *multi-stability*, is an inevitable mathematical consequence of a certain class of network structures because an attractor state is a type of solution in the equations that describe the coordinated changes of expression of the genes in the GRN – the network dynamics. However, a key idea is that not every attractor will represent a biologically observed enduring phenotype, such as a cell type. As Kauffman first noted (Kauffman, 1969, Kauffman, 1993), in a GRN of thousands of genes, there could be by mere mathematical necessity many more attractor states than the number of stable cell phenotypes ever needed. This important result will play a central role in our argumentation for atavistic attractors (see section 5).

A multi-attractor system, such as the GRN, has a broader epistemic implication because it provides a formal explanation for how the regulatory interactions between genes in the GRN can result in the departure from the 1:1 genotype-phenotype mapping. This "bottom-up"-explanation, based on dynamical systems theory, obviates the need for invoking "epistasis". The idea of epistasis is a phenomenological ("top down") *ad hoc* explanation introduced by geneticists to account for the departure from the simplistic, often tacit, *a priori* assumption of independent action of individual genes – a picture that is by default not assumed in the view of GRN as dynamical systems. The notion of attractor states that afford a single genome the capacity to encode a variety of stable (inheritable) cell phenotypes also obviates the need of invoking the (problematic) concept of epigenetic marks in explaining the stability of gene expression profiles of cell types. Epigenetic marks, such as DNA methylation and covalent chromatin modifications, would then merely represent the consequence of regulatory interactions imposed by the GRN, perhaps affecting the local kinetics of transcriptional activity of individual gene loci. The "intelligent" apparatus that computes the gene expression profiles is the GRN, wired by evolution to shape a landscape topography that governs tissue development and homeostasis.

4.2 Interpretation of Waddington's epigenetic landscape

In summarizing the above theory, we can go back to Waddington's epigenetic landscape of which the most famous form (Figure 1) , was only presented around 1957, almost 20 years after its initial conceptualization (Waddington, 1957, Waddington, 2012). We consider the marble in his landscape as representing a network state through its position: a distinct cell phenotype implemented by a specific gene expression profile. Then its tendency to roll down to the bottom of the valley, seeking the lowest point represents the driving force to change the gene expression profile in seeking the most stable configuration that emanates from the gene regulatory interactions. The rolling of the marble recapitulates the changes in the gene expression profile of the respective cell as it "spontaneously" alters its network state in a "directed" manner, attracted toward the bottom of quasi-potential wells in implementing the most stable state it can achieve from a given initial position. This is consistent with the aforementioned formal property that the elevation that defines the barrier height between the valleys captures the mathematical property of "relative accessibility" of an attractor from another. Thus, the landscape topography embodies the particularity of the intrinsic constraints imposed by the GRNs'

interactions between the gene loci that dictate how the gene expression profile is allowed to and has to change. It visualizes the "inevitability" of drive and direction of cell phenotype changes from within, thereby meeting the afore-discussed criterion for a theoretical explanation of a natural process.

Cell types are the result of development, which is a dynamic process: cells change their phenotype in development and in exerting their tissue function in the adult. In the phenotype space they are attracted to the stable attractor states, and they switch attractor states when they switch phenotypes. The valleys and hills between them impose a fundamental characteristic of differentiation: the quasi-discreteness of cell types and the quasi-discontinuous nature (all-or-none) of phenotype switching. It is in the context of such switching between stable cell phenotypes that the "elevation" of a point in the landscape (a cell state) has a special meaning (Huang, 2012a, Zhou et al., 2012, Zhou and Huang, 2011). The height of hills that separate the valleys and the relative depths of the latter captures the "difficulty" or "effort needed" to go from one attractor to another. This is of practical importance: a given attractor transition, which corresponds to cell phenotype switching (e.g. cell differentiation) requires a distinct amount of "regulatory effort".

We can now begin to extrapolate the behavior of a cell on the landscape to all the cells of a developing tissue, or even, embryo. The "topography" of the entire landscape of a genome will determine where the zillions of cells, each harboring the same GRN and hence, the same landscape, and each seeking local minima – but starting at distinct positions and influenced by distinct network perturbations due to distinct external perturbations, will "end up". The cells will, driven by gene expression noise, "swarm out" on the landscape and be attracted to the various attractor states in which they will stay and express the stable cell type specific gene expression profiles. An animated cartoon illustrating the controlled diversification of cell phenotype during development as cells proliferate and expand on the landscape to occupy the predestined stable types epitomized by the attracting valleys is presented by Mosmann and colleagues (Rebhahn et al., 2014).

The attractor property has one consequence that may be counter-intuitive to those accustomed to the current culture of thought in which precisely working, clock-like molecular machineries and linear causation connect genes with cell behavior. Not only does the stability of attractor imply robustness in the sense of disturbance tolerance in a noisy world but it also predicts that there is not one unique but there are ***many paths*** that can be taken to arrive at the very same gene expression profile that defines a distinct cell type (Huang et al., 2005). This multiplicity of causal history is the root cause of the robustness of directedness of development in a broader sense - or in Waddington's words, of the capacity of ***buffering*** and ***canalization*** (Waddington, 1942b, Waddington, 1956). The landscape thus epitomizes a creative combination of robustness, needed for homeostatic stability of phenotype, and flexibility, needed for development.

4.3 Transitions between attractors, their accessibility and developmental trajectories

The constrained flexibility of switching between characteristic (biologically meaningful) phenotypic states is prosaically captured by the afore-mentioned transitions between attractor states that require some regulatory effort. If a massive perturbation or a specific signal changes gene expression substantially, that is, affects a large enough set of genes, it can push a network state (a cell) from one attractor state into a neighboring one. Once the hill crest that separates two valleys (technically, a *separatrix* between attractors) is crossed, the attracting force of the new attractor state will ensure the self-driven realization of that new stable gene expression profile of the destination phenotype: the cells rolls down to the bottom of the valley and implements a new phenotype. Thus, the landscape imagery, or the concept of the quasi-potential of cell states, introduces the central notion of ***accessibility:*** can a particular attractor readily or not be accessed from a given position? This concept naturally emanates from the landscape picture, and it will be central to our discussion on atavism.

In network-based models of development (Huang, 2011, Zhou and Huang, 2011) cartoonishly summarized here, the cells start from the pluripotent zygote state, mature and in doing so, move from (metastable) attractor to attractor, recognizable as intermediate, yet distinct cell stages – or immature cell types. The movement on the landscape down from the immature embryonic cell to the mature terminally differentiated cell type at the lowest elevation is driven by the GRN-governed gene expression state change, and describe ***developmental trajectories*** on the landscape. They can branch (technically: undergoing bifurcations in a mathematical model used to explain controlled cell type diversification). Waddington coined the term "*chreod*" ("necessary path") to describe the canalized descend to the cell type

valleys (Waddington, 1942a), a term which could well correspond to developmental trajectories – or more generally – dynamical trajectories on the quasi-potential landscape.

For mathematical reasons related to the robustness of the expression level of thousands of genes, a cell attractor switch often requires a perturbation of thousands of genes in the network. Conversely, one often observes that rather non-specific perturbations that influence large sets of genes also can trigger a transition into a very specific state because precisely of the robustness or the attracting property of the destination state. It suffices to just "land" anywhere in the basin of attraction of the target cell state and the specific gene expression profile of a biological cell phenotype with self-organize as the cell descend to the attractor state. This explains why non–specific agents, such as solvents, or compounds that modulate DNA methylation and histone modifications, and hence affect the expression of thousands of genes across the genome, can trigger very specific differentiation events (Huang et al., 2005, Huang, 2001). Conversely, natural hormones and growth factors whose biological function is to induce differentiation, are "wired" by evolution to modulate, via signal transduction cascades, the expression of that specific set of genes, which collectively can mount the regulatory effort needed to counter the attracting force of an attractor, overcome the energy-barriers of the quasi-potential landscape, allowing the cell to enter into another one, thereby switching an entire gene expression profile.

Since the genome encodes the topography of the landscape it controls the developmental trajectories of cells and the accessibility of attractors, and thereby, ultimately, the ***relative ratio*** of the various stable cell types which determines tissue composition. It is important to remember here that despite the cartoonish image of development with cells rolling down, multiplying and filling up the attractors on the epigenetic landscape, the latter has a formal basis that links developmental cell phenotype change to the genomically encoded wiring of the GRN that orchestrates changes in gene expression profiles, affords attraction to the stable, physiological ones and also give rise to avoidance of incoherent, unstable ones. The landscape topography thus is a mirror of the genome's regulatory activity that establishes the developmental potentials – it capture the entire theoretical behavioral repertoire of cells. This will be important when we return to atavism. But first, we need to take a next level of abstraction: from cell type to tissues.

4.4 From cell type attractors to tissue attractors

In the second step of a formal conceptualization we will extend the landscape idea, in which each valley represents a cell phenotype, to a "higher level" landscape in which each valley represents an entire tissue or organ. This concept is much less well-developed but computer simulations of large arrays of coupled (identical) virtual GRNs, representing populations of interacting cells (Serra et al., 2010), have shown the existence of ***tissue attractors.*** Instead of stable gene expression profiles representing attractor states in the space of all possible gene expression profiles, the patterns of interest now are the configurations of cell populations that exhibit a stable composition (relative abundance) of various cell types. Stability analogously implies here: If one cell type in a given tissue, say the endocrine progenitor cells in the pancreatic gland, increases above a certain ratio in comparison to the exocrine cells, there will be a force that will restore the normal cell-type ratio.

How do stable cell type compositions of tissue arise, how are they maintained, and what are the restoring force that maintain the cell type ratios of a given tissue? It is evident that fundamentally, the relative abundance of cells must be sensed and that this requires cell-cell communication: some kind of quorum-sensing mechanism, as seen in micro-organisms, must be at play to maintain not only the number of cells but also the correct ratio and absolute numbers of cell types and keep them at the appropriate spatial position. In the previous section we explained how the shape of the landscape, in which the relative "depth" and size of potential wells or valleys, reflects the relative robustness (depth) and accessibility of attractors, and thus controls their relative occupancy. But the numerical occupancy of individual cell state attractors is further modulated by additional factors: cell-cell communication and external factors, which influence tissue-level processes, the relative survival and proliferation capacity of cells in various attractor states, transitions between attractor states. In addition, for the tissue the spatial regulation as to what cell phenotype a cell expresses at what position, must be considered as well as the relative survival/proliferation of cells depending of its tissue neighborhood (cells, matrix, soluble growth factors) and migration. These tissue-level cell behaviors are part of a broader developmental program but also linked to cell phenotype and hence to the GRN which determine which genes are expressed in what cell state. For instance, one type of cells may be in an attractor *A* that defines a gene expression profile that instructs the cells to express

and release a particular cytokine whose cognate receptor is expressed in another type of cells in attractor *B* as part of their attractor-defined gene expression program.

In summary, a complex *network of communication interactions between cells* along with a set of elementary behavioral programs of cells that are modulated by said communication and are robustly implemented by the attractors in the GRN of the individual cells, including proliferation rate, phenotype conversions and migration, determine the tissue patterns. Much as gene-gene interactions and gene expression dynamics of the GRN settle in stable gene expression configurations, so do cell-cell interactions and cell behaviors, which establish a population as a dynamical system, settle in stable ***tissue attractors***, defining the stable cellular pattern of a tissue.

This concept of tissue attractor is still in its infancy but is actually nothing more than a formal integration of a network that developmental biologists have been studying: the network of interactions between cells via soluble as well as solid-state interactions, such as shared extracellular matrix and the mechanical interactions in the morphogenesis of ordered tissue structures and organs. The very idea of tissue attractors adds a hierarchical dimension to Waddington's concept of epigenetic landscape that integrates two nested networks: the gene-gene and the cell-cell network. In this more encompassing framework, the valleys in the landscape represent the various tissues and organs of an organism rather than cell types.

The self-organizing tissue attractors unite, again, robustness and flexibility. They afford a piece of tissue some homeostatic stability and could be understood as the formal basis for the idea of the much discussed by elusive concept "morphogenetic field" in developmental biology.

Then, in a more encompassing framework of a biology that seeks to reduce phenotypes to the genome, development of tissues and organs is the extended unfolding of genomic information, via a first level of GRN dynamics that produces cells of distinct and robust types defined by gene expression profiles (our first conceptualization). The cells then engage via cell-cell interaction in a second level network (the second conceptualization) to generate robust tissue patterns defined by composition of cell types, their numbers and their location. This multi-scale dynamics of an intrinsic genomic program is of course influenced by environmental signals or (noisy) perturbations that can impose the switching of attractor states in the networks at both levels to affect developmental outcome. Thus, this framework is a formal basis for the departure from genetic determinism.

5. Evolution modulates the shape of the epigenetic landscape via rewiring the regulatory network

The landscape and attractor concepts offer a convenient formal tool to think about evolution of development and with it, the process of producing coherent, complex tissues and organs that however can sometime be inappropriate – atavisms. The genome determines the epigenetic landscape; environmental factors that affect the interaction strength can temporarily fine tune it, e.g. facilitating the access to a given attractor state by lowering the hills between the valleys (Huang, 2013). But the genome defines the basic landscape topography: existence and position of attractor states, and their relative depths and size and accessibility. Within the framework of the genomic landscape then innovation of new phenotypes in evolution is equivalent to the growth of the epigenetic landscape and inclusion of new valleys, that is, the addition of new attractors as the GRN is expanded by genes and interactions.

Since a genome maps uniquely into a landscape topography (modulo transient environ-mental modifications of its fine structure), the growth of the landscape must occur via an alteration of the GRN architecture. Such rewiring of the GRN and changes of the regulatory interaction logics in turn embody the actual consequence of genome evolution by genetic mutations and other genomic alterations.

Specifically, changes may include addition and deletion of new genes or alteration of regulatory interactions between gene loci (e.g. by mutations in DNA binding domains of transcription factors or their target site sin promoter), thereby altering the GRN. The ensuing distortion of the landscape topography reflects the consequence on the dynamics of the network and affects the developmental trajectories – or Waddington's chreods (see above). Thus, in this network view, evolution of development through genome evolution acts by altering the topography of the quasi-potential landscape. Concretely, these modifications may involve the creation of new attractors to represent new cell types, and the carving of new developmental trajectories in the landscape to channel some developing cells into these new attractor states.

Thus, evolution not only creates new phenotypes, but perhaps predominantly performs "fine sculpting" –much as rivers shape a landscape – to ensure smooth and efficient trajectories, in accordance with the developmental need for specific proportions of distinct phenotypes and the timing of their appearance. As we will propose below, this function also includes preventing developmental trajectories from being diverted into some "side valleys" – the attractors that existed purely for mathematical reasons and are never used (accessed) or have been used in the past but avoided by the newly evolving chreods that lead to new attractors (discussed in section 6).

The above mental cartoon of evolutionary change of the landscape helps to conceptualize a dual challenge long discussed in evolution biology. As the landscape grew due to GRN expansion by new genes (e.g. following genome duplication events), and new attractors are created that encode new genetic programs representing new phenotypes (such as cell types and tissues), evolution also must guarantee their accessibility if these novel attractors are to be manifest as phenotype and exposed to natural selection. If the new phenotype confers a significant advantage, further evolution will reshape the landscape, such as to modulate the upstream developmental trajectories to increasingly facilitate the access during development to these new attractors that encode biologically meaningful gene expression profiles.

The above picture is plausible – but what is the formal support for reshaping of the landscape by rewiring of the GRN? Computer simulations of rewiring in large, generic GRNs by Aldana et al. (Aldana et al., 2007) show that such moderate reshaping of the landscape that distorts but conserves attractor basins is indeed a generic change provided that the GRN belongs to a particular class of network architectures called "critical networks", characterized by relative sparse connectivity and some particular regulatory. Computational models of network evolution as well as bioinformatics analysis suggest that existing evolved GRN indeed appears to belong to the class of "critical networks" (reviewed in (Huang, 2009, Aldana, 2003)). Such network architecture are said to be "structurally stable" (Huang, 2009) in that random rewiring of the GRN in simulations, which represents the effect of genetic mutations in trans-regulatory factors or cis-regulatory elements, very rarely has catastrophic consequences on the landscape topography. Thus, the landscape can "buffer" genetic rewiring of the GRN and more or less maintain its shape. Indeed, simulations show that as a consequence of random rewiring, occasionally new attractors are added or old ones deleted while the existing landscape is typically only mildly distorted.

The most common consequences of random rewiring consist of either slight shifts in the position of attractors, which would map into minor modification of the gene expression profile encoded by that respective attractor, or of changes in basin structure (size and barrier heights), thus modulating the probability ("ease") of transitions into that attractor (Aldana et al., 2007). In other words, mutational rewiring in attractor landscapes of critical networks seem to have a high probability to modify phenotypes rather mildly, and most notably, they can modulate developmental trajectories, such as rate and timing of cells' entry into particular attractors and the specific conditions that favor these transitions. This property has been considered to be central to evolvability, and is a characteristic feature of critical networks (Aldana et al., 2007, Torres-Sosa et al., 2012).

Interestingly, whole-genome sequence comparison now reveals that most evolutionary changes that account for the gross anatomy phenotype differences between related species - such as among mammals, or even vertebrates - are not due to changes in the sequence of effector genes that encode proteins with defined function directly manifest at the phenotype level - such as enzymes and structural proteins - but rather affect regulatory interactions since much of interspecies genome sequence variability lies in regulatory sequences (Prud'homme et al., 2007).

6. Atavistic attractors as latent attractors that encode ancient traits

The formal relationship between GRN structure (which is the target of mutations) and the topography of the epigenetic landscape that guides development of cells and tissues offer a tool to conceptualize the principles through which evolution modulates development. We can now take a step closer to the central postulate of this article: namely, that atavistic traits, that is, ancient tissues or organs, are encoded by "hidden attractors" in the epigenetic land scape which are normally (in physiological development) not accessible. The principles are summarized in Figure 3. The questions we now seek to answer are: if atavistic traits represent programs encoded by hidden, latently present attractors, why do latent attractors exist in the first place, how are they accessed, and why do they encode ancient traits?

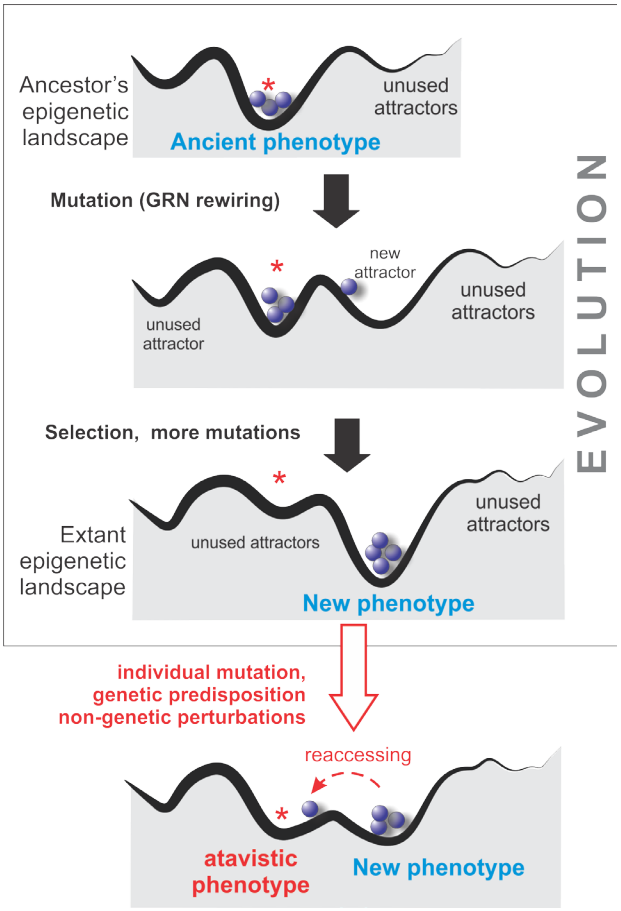
One central point is that development of atavism is not a *de novo* creation of developmental programs but much simpler, the uncovering of latent programs. While such verbal circumscription of a natural phenomenon in abstract terms, such as the notion of presence in a "latent" form, is widely used, we have now a formal basis, in terms of molecular and mathematical concepts, that in principle can explain a latent structure and the relative ease of producing a complex structure under particular circumstance by utilizing said latent structure.

Specifically, we can now readily appreciate that certain mutational distortions of the landscape could lower some epigenetic barriers that evolution has erected (via selection of suited network-rewiring mutations) to shield away unused attractors, and allow for developmental trajectories to enter these hidden attractors (see 6.1). These latent attractors may encode specifically ancient traits (see 6.2). The pathological access to hidden-away attractors can be manifest in degrees: creating an obligate, inevitable sidetrack of the developmental trajectory or merely increasing the probability for accidentally entering and occupying the atavistic attractor. The latter case would be manifest as variable penetrance in genetics or in a requirement for additional contributing genetic or environmental factors to implement the abnormal trajectory to the hidden, atavistic state.

But the concept of gaining access to hidden attractors, whose existence is, as we have seen, mathematically quasi-inevitable, only shifts the explanation of atavism. For it opens the central question: if indeed many abnormal, not-to-be used attractors exist, why do they exist and why do they specifically encode ancient phenotype? The answer that we propose has two parts and naturally follows from the above concepts of the evolution of the quasi-potential landscape as the genome and its associated GRN evolve.

6.1 The existence of latent attractors on the epigenetic landscape

First, we note that the normal epigenetic landscape has many more attractors than actually required to produce all cellular and issue phenotypes. Remember that the quasi-potential landscape is a direct mathematical consequence of the GRN wiring diagram and thus, subjected to mathematical constraints that dictate the existence of attractor states: attractor are solutions of equations that describe the dynamics of the gene expression profiles driven by the gene regulatory interaction in the GRN.



Fiure 3. Evolution of the epigenetic landscape, atavistic attractors and re-access. Schematic sections through the quasi-potential landscape explained in Figure 2. Evolution (within the box) is the mutational rewiring of the GRN, which alters the shape (topography) of the epigenetic landscape. Evolutionary innovation, e.g. of a new cell type or tissue type, is due to the change of the landscape such that a new attractor is generated that is also readily accessed during development and encodes the new a cell or tissue state; at the same time, phenotypes encoded by old attractor may not be needed anymore (red asterisk). However, the old attractor, which is not needed anymore as simulations of network evolution show, is often not deleted in the course of evolutionary change of the landscape. Instead, the developmental chreods are simply re-channeled such that the ancient attractor is no longer accessed for topographical reasons, e.g. there is high potential barrier that separates it from the chreods of normal development. Outside of evolution (bottom), for a given individual, the accidental occupation of such an unused but latently present attractor can happen either as a consequence of genetic mutations that affect GRN dynamics so as to lower a quasi-potential energy barrier or following strong non-genetic perturbations, manifest as developmental plasticity, that pushes the developing cells over a high barrier (dashed arrow). Either way, undesired occupation of the ancient attractor results in the activation of the gene expression programs encoded by these ancient attractors and, it is manifest as atavism.

Thus, as already proposed by Kauffman (Kauffman, 1969) and later refined by Huang (Huang, 2001), a given network produces many more attractor states than ever

visited by cells during development and used to encode organismal function in the adult. Thus, hidden in the "dark space" of the vast epigenetic landscape which contains all possible configurations of activities of all gene loci of the genome, far from the regions through which developmental trajectories pass, inevitably there exist uncounted numbers of potentially stable configurations. Only the attractor states that are occupied by cells during development or in adult life will be manifest as phenotype and exposed to natural selection. Only then can selection act, whose substrate is the random mutational rewiring of the GRN, which in turn shape the landscape – quite conservatively because of above discussed mathematical constraints.

With the presence of *excess attractor states* on the epigenetic landscape (which by definition require quasi-potential energy to exit from), there is ample danger that the developing cells are side-tracked and "get sucked" and then get "stuck" in inappropriate attractors on their way down to the physiological attractor that encode functionally important phenotypes, such as specialized cell types, that have been shaped by natural selection. In other words, development could accidentally enter "side valleys", due to molecular noise, which causes cells to wiggle around their trajectories. Then perhaps one task of evolutionary fine-tuning would be the rewiring of the GRN to sculpt the landscape such as to minimize the chance such erroneous departure from the normal developmental trajectories because of the many unwanted stable states that lurk in the dark along the way. The carving of developmental "highways" into the landscape that ensure efficient and secure reaching of the physiological attractors by the developing cells and tissues could be achieved by increasing the hills to separate away the inappropriate attractors to guide the cells along trajectories that pass the developmentally correct intermediate states. This is plausible because such modifications of access are much more readily implemented by (random) rewiring of the GRN than the complete elimination of attractors. It is in this sense that Waddington's chreods are robust – and that development is, in his words "canalized" and "buffered" (see Section 5).

6.2 The specific atavistic nature of the program encoded by latent attractors

Second, if atavism is the undue access to one of the many latent unphysiological attractors, which are the inevitable mathematical by-products of GRN dynamics and from which normal development is shielded, then we need to explain why these unused stable states *specifically* encode ancient developmental programs. Why do the hidden attractors represent any meaningful, biologically coherent gene expression profiles in the first place?

In the evolutionary expansion and modification of the epigenetic landscape that underlie increasing organismal complexity in terms of invention of new cell types and tissues, not only are new attractor states added. The change of the landscape that accompany organismal changes also requires the cessation of use of existing attractors, for some tissue structures are no longer needed in the changing organism. It follows naturally from the afore-discussed relative ease to modulate access to existing attractors as opposed to affecting their very existence, that mutations are much more likely to prevent entry into certain attractors, by increasing the height of quasi-potential barriers – the hills that separates them from the chreods.

Thus, the mathematically grounded inevitability of excess of attractor states in a theoretical, complex randomly wired (critical) network is in the case of a biological, evolved GRN also epitomized by the existence of abandoned previously used attractor states. In other words, since it is evolutionary cheaper to block access to unnecessary attractors than to delete them, the evolved epigenetic landscape may contain large regions in which relatively stable states that represent ancient but "discarded" programs are abundant.

Finally, the idea that the genome harbors evolutionary baggage is of course not new. Traces of our ancient history are amply evident in the genome sequence (Rasmussen et al., 2010). Pseudogenes and sequence homologies across large taxa are the footprints of our evolutionary history. But here we go beyond the detection of localized footprints of evolution. We propose an integrated picture of the genome-wide GRNs and explain evolutionary remnants at the level of developmental programs not of the genome. We seek to account for the reappearance of complex phenotypes that are recognized as "throwback". By invoking the formal concept of hidden attractor states and their accessibility on the epigenetic landscape an explanation is proposed for the aberrant development of complex, well-organized traits with relative ease, yet at relative low frequency.

7. Specific examples seen in the light of atavistic attractors

We have in the previous sections presented in detail the general principles that explain the unavoidable existence of atavistic attractors and how they could be accessed "accidentally" during development – due to mutations that lower the entry barrier or to environmental perturbations that push cells and tissue across the latter. But what are concrete cases and how does it happen in terms of specific molecular pathways? Here we will visit several scenarios of observed spontaneous and experimental atavistic phenomena, from the perspective of the epigenetic landscapes and attractor states.

That atavism can be triggered by mutations is documented by several examples. Figuera et al. studied how the mutation Xq24-q27.1 region of the X chromosome evokes hypertrichosis (excess hairiness) (Figuera et al., 1995). DeStefano et al. suggested a role for FGF13 in hair follicle growth and in the hair cycle (DeStefano et al., 2013). The genetic basis of atavisms has been demonstrated in selective breeding experiments on guinea pigs. It has been shown that spontaneous mutation caused development of supernumerary and nonfunctional fourth toe from a single guinea pig. Later on, polygenetic basis of inheritance of this atavistic digit was shown (Hall, 1984).

Mutations in the genome rewire the GRN and, as explained above, can facilitate the deviation of the developmental trajectory from the "safe" regions of the epigenetic landscape (tested by evolution) if they lower the quasi-potential barriers that may have evolved to constrain phenotypes within the normal chreods. The reduced barrier height may allow traits to take the "path not taken" on the epigenetic landscape and enter ancient attractors. Note that these attractors, being unoccupied, hence not expressed, have long been shielded from natural selection. Therefore, mutations in the genome may affect the particular gene expression configuration of these unused (but not that of physiological) attractors leading to an altered, non-functional atavistic trait, and thus a departure from the original phenotype.

In contrast to mutation-associated forms - such as hind limbs in whales, extra toes in horses, extra nipples in humans, supernumerary teeth (hyperdontia: extra teeth that develop in supplement to the dentition), oral vestibule, pre-lacteal teeth, paramolar cusps/teeth in mammals, and the tooth glands in reptiles - other miscellaneous atavisms have been regarded as spontaneous atavisms (Hall, 1995, Gupta et al., 2015, Iurino and Sardella, 2014, Peterkova et al., 2006, Peterkova et al., 2005). Following the principles discussed, "mutation-less" transition into the atavistic attractor can thus take place by stochastic fluctuations of the network state. Such a process is captured by, and was a motivational factor in the development of theories of the quasi-potential landscape. In theory, if the stochastic fluctuations of molecules that specify a given network attractor state *A*, due to molecular noise, at some point (by chance) reach a sufficiently high amplitude, such that it exceeds the quasi-potential barrier ΔU (see FIG. 3) of the hills separating that state from the neighboring attractor state *B*, this could result in the stochastic, spontaneous transition of a phenotype from that attractor into the nearby one. Thus, atavistic transformations might occur when at some point in normal development some cells on the physiological developmental trajectory passes close to a region that is normally shielded by high quasi-potential barriers and that contain atavistic attractors, randomly, with no apparent cause, "spills over" to these ancient, hidden attractors. Once the potential phenotype encoded by the ancient attractor is expressed by the occupying cells, a parallel development of entire atavistic programs may ensue.

But if a random chance event can (rarely) trigger the unfolding of entire suppressed programs by the crossing of quasi-potential barrier by a few cells, so could this stochastic process be enhanced by environmental signals. Modulating the strength of regulatory interactions can, as can be readily shown mathematically (Huang et al., 2007, Mojtahedi et al., 2016) (transiently) affect heights of barriers that separate two attractors. This is conceptually similar (but fundamentally distinct in terms of the physics) to biochemical catalysis by enzymes, and can promote rare transitions – obviating the need to depend on mutations.

In line of our thinking based on a formalism, Tomic and Meyer-Rochow have emphasized that "The induction of atavisms is hardly an induction of a novel creation (Tomic and Meyer-Rochow, 2011): the induced structures have already been refined in a gradual, laborious manner over the course of some earlier evolution". As an experimentally induced form of atavism, teeth in birds provide a close examination opportunity for atavism (Kollar and Fisher, 1980).

Odontogenesis requires epithelial and mesenchymal interactions. The cells are derived from ectoderm of the first branchial arc and the ectomesenchyme of the neural crest. Interaction allows the epithelial part to form outer layer of enamel and mesenchyme is responsible to form inner layers (dentin, dental pulp, attachment

to bone, bone, etc.). In 1980, Kollar and Fisher grafted sixteen to eighteen day-old mouse mesenchyme, which were taken from the region where first molar teeth form, alone into the suitable space of anterior chamber of eyes of adult nude mice (Kollar and Fisher, 1980). Dentin was not developed. However, when they combined that mesenchyme with an epithelial tissue from the first and second gill arches of a five-day-old chick embryo, they observed dentin. Thus, they proved that under appropriate conditions, the lost odontogenic capacity of avian ectomesenchyme can be regained since the oral epithelium still has odontogenic capacity (Kollar and Fisher, 1980). This stunning experiment proved and convinced the evolutionary biologists of, as Gould said, "the potential structure that chick epithelium has encoded for sixty million years but has not expressed in the absence of dentin to induce it" (Gould, 1994). Regaining the lost odontogenic potential in birds has been repeated by several experiments (Cai et al., 2009, Chen et al., 2000, Fuenzalida et al., 1990, Harris et al., 2006, Kollar and Fisher, 1980, Lemus, 1995, Mitsiadis et al., 2003, Wang et al., 1998). With the concept of latent attractor as by-product of GRN dynamics and of their atavistic nature as a result of the way the epigenetic landscape changed during evolution, we now also can articulate the formal and molecular principles to support these verbal assertions. These experiments also are in line with the above account that mutational rewiring of the GRN is not necessary but that environmental signals, which are complex and poorly understood and can only be replicated by transplanting entire supporting issues, can unleash the phylogenetically hidden developmental potentials. The need for a complex set of signals that come as a natural "package" in the form of the physical presence of an inducing tissue is also consistent with the need for broad combinatorial perturbations of the nodes in a network to cause an exit from an attractor state.

An interesting example in birds that is related to atavism is the induction of brown fat in avian cells. Birds lack brown fat, a tissue that generates heat in eutherian mammals. However, cells nearly identical to brown fat adipocytes could be induced from chicken mesenchyme in vitro by overexpression of a single gene (Mezentseva et al., 2008). Although this is strictly not a form of atavism, since it is not likely that ancestors of birds possessed brown fat tissue (which is only present in a subset of mammals), this observation suggests that a particular attractor state in the GRN of (a subset) of vertebrates that encodes for a complex cell phenotype related to that of brown fat cells is inherent in the genome shared by birds and mammals but has not become accessible in those taxa that do not possess brown fat.

Taking a more encompassing view, the examples of atavism (or related phenomena) also highlight how the concept of the quasi-potential landscape, in which atavism is caused by the undue access, from the developmental trajectory, of preexisting but normally inaccessible attractors, unites genetic and non-genetic causes of atavism. By offering such an integrative framework this traditional dualism is relegated to a mechanistic detail.

8. Conclusion

Hall described three major conceptual, comparative anatomical and developmental biological criteria of atavisms, and here we show that the concepts of GRN and attractors on an epigenetic landscape, and in particular, the idea that of atavistic attractors can explain these criteria (Hall, 1995):

(1) *Atavisms show a high degree of phenotypic resemblance to a trait of a predecessor* (i.e., atavism is not found in parents or immediate ancestors). This is readily explained by the evolution of the epigenetic landscape that governs development: unused phenotype traits to be eliminated are not actually eliminated by deleting the genes that govern there development, as one would think in the common view of a 1:1 genotype-phenotype mapping. Instead, only developmental trajectories (chreods) are modified by reshaping the epigenetic landscape such that the attractor states that encode these phenotypes are not accessible anymore.

(2) *Atavism appears as a fully developed character in adult life stages.* We apply here the formalism of the landscape and attractors not only for cell types (stable gene expression pattern) but for entire tissues (a characteristic, stable cell type composition) in which we propose "tissue attractors"; thus once accessed, a latent atavistic program can unfolded and govern the development of entire coherent multicellular tissues or organs.

(3) *Atavism is marked by infrequent occurrence across populations, usually in one or a few individuals.* The latent attractors that are not accessible require quite particular sets of modification of gene expression to surmount the quasi-potential energy barrier that prevents their access from the physiological chreods. However, once the rare perturbations required for overcoming the energy barrier have directed development of a set of cells into such attractors, their ensuing development to a coherent ancient tissue is self-organized. This concept explains the counterintuitive combination of rarity of the apparently spontaneous occurrence of some structures with

the complexity of the organized order once the rare conditions are met. One can also argue that the rarity of atavism is a consequence of the possibility that the noise-driven accidental deviation from the physiological trajectories into unused attractors may have most of the time no consequences and would not be manifest in atavistic structures because such aberrant cells would simply die. This is plausible in view of the idea of the absence of optimization of gene expression programs of the unused attractors by evolution. The majority of deviant cells that have moved away from the physiological chreods may end up in unused attractors that do not encode any coherent viable and robust tissue state.

(4) *No organism can display an atavistic structure that was not previously found in its ancestry.* This again is consistent with the idea that most unused attractor exist only as mathematical by-product and do not encode any meaningful gene expression profile that govern viable programs. The phenotypic manifestation of an accidentally occupied unused attractor is possible only because the associated gene expression program had once been utilized and exposed to natural selection until they were made inaccessible.

Finally, we note that while the conceptual framework propose here is based on "first principles" of dynamical systems theory, it still lack the specifics. Nevertheless, such a view that emphasizes concepts and basic principles that *must* be obeyed, in some form offers a counterweight to current approaches in modern biology that seeks the specific molecular "proximate" mechanisms without much effort in establishing consistent, integrating principles. Proximate explanations are not constrained by the inevitability (in whatever from) of first principles. Many of the concepts used here stem from simulations of large abstract toy models, randomly wired gene regulatory networks. But as the resolution of our maps of gene regulatory networks increases, we may soon be able to erect more specific hypotheses for a given atavism and refine the generic principles presented here. Until then the generic concepts serve as a "tool of thought" as Waddington (Waddington, 1977) liked to say that may help to organize not-so intuitive observations surrounding atavism into a coherent framework.

Acknowledgments

S.H. acknowledges funding support of his laboratory by the National Institute of Health (R01GM109964, 2P50GM076547-06A1) and the Institute for Systems Biology, Seattle. The authors would like to thank Dr. Stuart Kauffman for many inspiring discussions.

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