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A linear-encoding model explains the variability of the target morphology in regeneration

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A fundamental assumption of today's molecular genetics paradigm is that complex morphology emerges from the combined activity of low-level processes involving proteins and nucleic acids. An inherent characteristic of such nonlinear encodings is the difficulty of creating the genetic and epigenetic information that will produce a given self-assembling complex morphology. This 'inverse problem' is vital not only for understanding the evolution, development and regeneration of bodyplans, but also for synthetic biology efforts that seek to engineer biological shapes. Importantly, the regenerative mechanisms in deer antlers, planarian worms and fiddler crabs can solve an inverse problem: their target morphology can be altered specifically and stably by injuries in particular locations. Here, we discuss the class of models that use pre-specified morphological goal states and propose the existence of a linear encoding of the target morphology, making the inverse problem easy for these organisms to solve. Indeed, many model organisms such as *Drosophila*, hydra and *Xenopus* also develop according to nonlinear encodings producing linear encodings of their final morphologies. We propose the development of testable models of regeneration regulation that combine emergence with a top-down specification of shape by linear encodings of target morphology, driving transformative applications in biomedicine and synthetic bioengineering.

1. Introduction

Large-scale morphology, including anatomy and patterning, is considered an emergent property of developing and regenerating organisms. There is no blueprint stored in the zygote; instead, a nonlinear encoding based on genetic and epigenetic networks drives development through the expression of diffusive [1] and reactive [2] biochemical signals [3–5], together with the mechanical and electrical properties of living cells [6–8]. Morphologies are high-level outcomes that unfold by the action of these networks that involve large numbers of concurrent low-level cellular mechanisms and their nonlinear interactions [9–13]. As in development, biological regeneration of organs, such as amputated amphibian limbs, involves the control of a complex network of genetic, biochemical and bioelectrical signals [14–17]. Indeed, many mechanisms necessary for regeneration are also present during development, and it is often stated that regeneration recapitulates morphogenesis [18,19]. Regeneration, therefore, is also commonly regarded as an emergent process controlled not only by a stored blueprint of the overall form, but also by nonlinear genetic encodings that control the action of low-level cellular mechanisms.

However, recent advances in developmental biology have revealed that, during development, low-level cellular mechanisms produce morphogenetic fields that prepattern the embryo; these serve as instructional information to which individual cells respond to form the resultant morphology [20–22]. These prepatterns are based on morphogen concentrations created by genetic networks and diffusion or reaction–diffusion mechanisms [23,24], electric

gradients created by electrical circuits formed within and between cells [21,25] or mechanical forces exerted and produced by the living tissue itself [6,26–28]. Thus, although formed by indirect low-level mechanisms during development, these fields and prepatterns represent a one-to-one encoding (a blueprint) from which further cellular mechanisms create the final morphology.

Moreover, the regenerating large-scale morphology of certain model organisms can be predictably altered, which suggests that the underlying mechanism of these regenerative processes is not based on a nonlinear encoding. As we review in the following sections, the target morphology—the shape to be restored during a regenerative process—of deer, planaria and fiddler crabs can be modified in a localized way through specific injuries or pharmacological treatments. The new regenerated morphology is either permanent or can last for several cycles of regeneration, without the need of reapplying the specific injuries or drugs that produced the change in the first place. Importantly, changing a nonlinear encoding to emergently regenerate a new shape or pattern represents a very hard inverse problem that cannot be efficiently solved [29], which discards the involvement of nonlinear genetic encodings in these regenerative systems. For example, given a genetic network (a nonlinear encoding) regulating the developing of a specific morphology, it is very difficult to determine what genes or links should be changed in order to produce a non-trivial desired specific change in the morphology, such as adding an ectopic limb or organ. A simple analogy can be made with ant behaviour. Each individual ant is following local rules about pheromone signals, and no single ant knows anything about the shape of the resulting anthill. Modelling the time evolution of such a system forward, it is easy to see how massively parallel execution of nonlinear rules can give rise to surprising and complex outcomes [30,31]. But, how would one modify the simple rules guiding each ant if one wanted the resulting anthill to have one extra lateral chimney?

This problem stands in sharpest focus in regenerative medicine, where we are faced with knowing which genes to tweak and how, in order to recreate a missing arm or an eye. While molecular pathways have made great strides in regulating the differentiation of stem cells into specific lineages, the incredible complexity of genetic and biophysical networks is a potent roadblock to the development of interventions that make desired changes at the level of anatomy (e.g. grow back the index finger, enlarge the lobe of one lung or rearrange craniofacial morphogenesis to repair a birth defect). A few examples of such anatomical change, leveraging developmental modularity, exist [32,33]. But, in general, the mathematics of nonlinear interactions in such complex emergent systems places fundamental constraints on our ability to know which gene products must be tweaked so that, when all cells carry out the resulting genetic network, a specific change of large-scale anatomy will result.

By contrast, in a system based on a linear encoding, the strategy would be different. For example, it is trivial to deduce from a one-to-one encoding (a blueprint, the simplest case of linear encoding) the changes necessary to specifically alter the morphology, because a change in the blueprint directly translates into the same change in the morphology. Knowing how the target morphology is linearly encoded in the chemical or physical properties of cells, one could change this information directly, and then rely on individual cells to build the shape without trying to micromanage the process [34]. Many issues of evolutionary developmental biology are impacted by the possibility that such linear encodings are used in embryogenesis. Moreover, the challenges of biomedicine for traumatic injuries and birth defects require that we take seriously models that may greatly augment our ability to direct growth and form at will. Finally, strategies for the bioengineering of novel hybrid structures in synthetic biology will be different depending on whether these linear encodings exist and can be manipulated.

While modern biology largely eschews anything that resembles the early theories of preformation, it must be remembered that regulative development, metamorphosis and regeneration have remarkable ability to *reach an anatomical goal state* despite considerable external perturbations of the number and locations of cells. Classical experiments [35] showed that early embryos can be divided or combined and give rise to perfectly normal animals [36]. During starvation, planarian flatworms continuously remodel and adjust organ sizes allometrically to precise proportions as available cell number is reduced [37]. In amphibian metamorphosis, artificial perturbation of tadpole facial anatomy becomes normalized into quite normal frog faces despite the fact that the organs start out in bizarre positions and must navigate around each other (in paths not predictable by evolution) to reach the correct frog face anatomy [38]. Tails grafted onto flanks of salamanders slowly remodel into limbs [39]. All of these are examples of cellular activity that is adaptively and flexibly controlled towards a target large-scale shape.

An increasing subject of inquiry in genetic circuits seeks to show that emergent features of gene-regulatory networks include the systems property of robustness [40]. However, this has largely not been addressed at the level of large-scale shape [41], and there is a dearth of models to explain how cellular activity is guided towards the specific anatomical outcomes when the starting states were significantly different from normal (ruling out hardwired actions). One tempting set of concepts for investigating such models concerns top-down [42–44] regulation (signals operating at the level of organ shape/size/identity, not cell behaviours), and implementation of algorithms that work towards specific goal states [45]. Such models often require the physical encoding of the target morphology.

In the next sections, we detail the target morphology variability exhibited by several organisms and discuss one-to-one and other linear encoding models that can explain this variability—a theme that has been out of favour for many years in the age of molecular cell biology. We show how these organisms are effectively solving an inverse problem—an achievement hardly possible with a nonlinear encoding, but trivial with a linear encoding. The experimental and theoretical evidence for the existence of a linear encoding of the regenerative target morphology suggests a rich and interesting research programme, which provides a necessary complement to the current roadmap for understanding self-assembly and repair of biological structures.

2. Variable target morphology in regeneration

The amputation of a salamander leg triggers a regenerative process combining growth and repatterning that restores the

Table 1. A summary of organisms in which the target morphology can be altered.

organism	regenerative part	target morphology	target morphology alteration
deer	antler	antler pattern	injury during regeneration
planaria	almost any body part	head, trunk, and tail regions pattern	amputation under GJC-blocking drugs
fiddler crab	chelipeds	handedness pattern	cheliped severance during development

original morphology [46,47]. As in most organisms with a regenerative capacity, the target morphology that this regenerative process creates is always the same: the original morphology of the wild-type limb. However, in some regenerative organisms, the target morphology that their regenerative process restores can be specifically altered through surgical manipulations or drugs. Among these animals are deer, planarian flatworms and fiddler crabs, whose characteristics are summarized in table 1 and detailed below. The most fundamental prediction of any linear encoding model is this: if a target morphology is linearly encoded and guides cell behaviour, then it should be possible to specifically change it, resulting in a stable change in the pattern to which the animal regenerates upon damage. This is indeed observed in a number of remarkable model systems.

2.1. Variable target morphology in antler regeneration

Antlers are deer appendages that cast and regenerate every year as extensions of the two permanent bony protuberances of the frontal bones called pedicles [48,49]. In general, only male deer grow antlers [50], following a cyclic process synchronized with the natural light cycle [51]. Initially, regenerating antlers contain a dense vasculature network and many sensory fibres that grow from the pedicle [52]. When growth stops, bone is formed in high quantities and the enveloping skin (velvet) dry and shed, leaving only the exposed solid bone [51]. Finally, the antlers are shed after the mating season, and a new cycle begins. The evolutionary adaptation of antler cyclic regeneration may be explained by the mechanical superiority of dry antler compared with wet bone in terms of elasticity, strength and impact absorption [53] and the difficulty for the body to maintain a junction between living and dead bone tissue [49].

Little is known about the control mechanisms of antler regeneration [50]. Stem cells located in a niche in the pedicle activate periodically, and are crucial for the regeneration of a new antler [50,54,55]. Hormones, such as testosterone and insulin-like growth factor I, are required for the growth of the pedicles and the development of antlers [50,51,56,57]. Research on local mechanisms of growth control has shown that retinoic acid, PTHrP/Indian-Hedgehog pathway, the canonical Wnt pathway and bone morphogenetic proteins are involved in the antler growth process [50]. Growing antlers are profusely innervated [58], and classical experiments have shown that electrical stimulation of the antler nerves during antler regeneration causes overgrowth and abnormal branching patterns [59–61]. Yet, the antler can regrow from a denervated pedicle, although smaller, lighter and with an altered shape [62,63].

The antlers' shape is incomplete during the first years of life; until maturation, the number of branches and total length increase with age, where the morphological variability decreases [64]. Because the morphology of the antler is species-specific, it is believed to be under control of genetic mechanisms [48]. However, experiments have shown that the antler target morphology can be specifically altered for several years owing to a single injury produced during regeneration—a phenomenon called *trophic memory* [65,66].

Figure 1 illustrates trophic memory in a white-tailed deer (*Odocoileus virginianus*) [56] and a Siberian wapiti (*Cervus elaphus xanthopygus*) [65]. Figure 1*a* shows three-dimensional reconstructions of computed tomography scans of the antlers of a white-tailed deer from year 5 to 8. The antlers regenerated normally in year 5 (first row), but, in year 6 (second row), an injury during the early developmental stages of antlerogenesis was suffered in the left antler. The injury altered the target morphology of this antler in that year, creating an atypical 'royal' (red arrow) instead of a single tine precisely in the location of the injury. This new target morphology was generated during years 7 and 8, producing a royal in the same location (green arrows) in the absence of any additional injury. In addition, the target morphology of the right antler was altered in a similar way, producing a royal in the reciprocal location during years 7 and 8 (blue arrows). Figure 1*b* shows the regenerated antlers (one side) of a Siberian wapiti during three consecutive years. During the first year shown, a slight cicatrize (red arrow) was produced by a cut off the dorsal portion of the germinative bud when the antler had reached nearly 40% of its normal length. Similar to the white-tail deer, this injury altered the antler target morphology: the following 2 years, the regenerated antler presented a new tine at the site of the original injury (green arrows).

Trophic memory was also observed in fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and moose (*Alces alces*) [65,66]. Stronger injuries, such a fracture in the pedicle, can cause stronger pattern alterations in the target morphology during the following regeneration cycles [59,66]. However, not all injuries produce trophic memory. For example, injuries near the end of the antler growth do not affect the antler development in the following cycles [65]. Remarkably, completely anaesthetized animals do not exhibit trophic memory either, regenerating the normal antler morphology during the following cycles after an injury [59,66], suggesting that some aspect of neural function [67,68] or bioelectrical communication among non-neural cells [69,70] is important for trophic memory to occur.

The implications of this phenomenon are profound for three reasons having to do with patterning information encoding in space and time. Spatially, the injury is made to a structure that is completely removed before next year's growth shows an altered pattern. This reveals that the modification induced by the wounding was not a local event, but was transmitted a long distance to the growth zones at the scalp. Second, as with the other examples discussed below, this is a true example of a

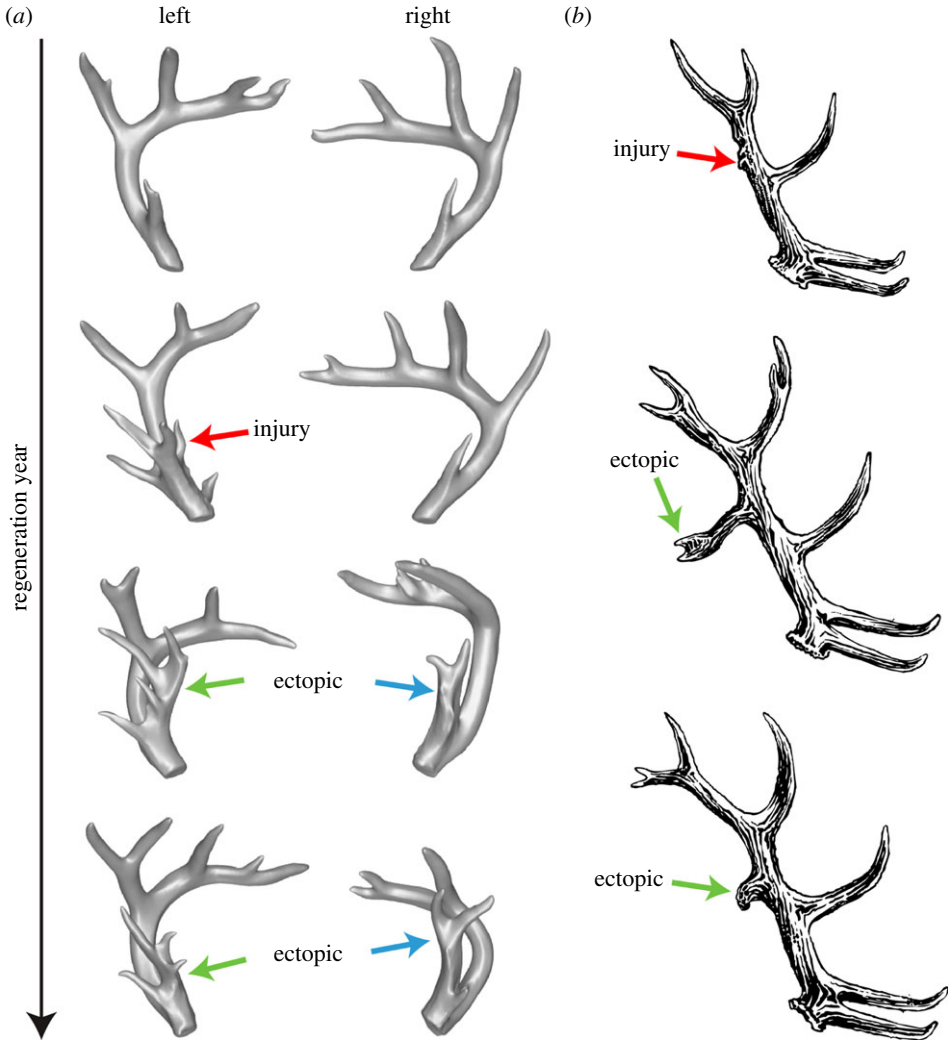

Figure 1. Deer antler variable regenerative morphology. (*a*) Using computed tomography scans, we reconstructed in three dimensions the shed antlers from a white-tailed buck from years 5 to 8. In year 6, the left antler suffered an injury during the early developmental stages of antlerogenesis, producing a 'royal' instead of the usual single tine (red arrow). This injury caused the alteration of the regenerative target morphology: in the following years, the left antlers regenerated the ectopic royal in the same location as the original injury (green arrows), and the right antlers (which were never injured) developed a less developed royal in the reciprocal location (blue arrows). (*b*) On a Siberian wapiti, a cut off the dorsal portion of the germinative bud when the antler had reached nearly 40% of its normal length produced a slight cicatrize in that year (red arrow). The injury altered the target morphology, producing during the following 2 years a new tine (green arrows). Diagrams in (*b*) modified after [65].

kind of memory, because months pass between the original insult and the altered growth—whatever change has occurred, remaining cells must remember to alter the growth next year. Interestingly, related memory of positional information has now been demonstrated in salamander limb regeneration [71] and adult human fibroblasts [72,73]. Lastly, the ability to recreate an ectopic tine *in the same place* within a branched complex structure each year provides an ideal illustration of the inverse problem. Without a linear encoding, cells would be stuck with the intractable challenge of determining how to change their local growth rules so that next year, an ectopic tine was created in, and only in, the correct three-dimensional location. Although it is not yet known to what spatial accuracy the positional information is kept (what is the resolution of this memory system), the cut could have been made anywhere along the branched structure, rendering it very difficult to see how purely local growth rules could be altered to produce the needed ectopic growth in the right place. Such a phenomenon is not at all predicted by any emergent paradigm or molecular pathway model. By contrast, a linearly encoded target morphology model accommodates this finding easily, because once the linear representation of the branched structure is changed to include an extra tine, subsequent years' growth will implement it. While this model system is relatively expensive, it is imperative to begin to investigate the mechanisms by which such branched morphologies can be stably encoded in tissue and the information altered by damage signals.

2.2. Variable target morphology in planaria regeneration

Planaria are flatworms with a complex bilaterally symmetric bodyplan, a brain allowing complex behaviours [74], and an outstanding regenerative capacity driven by a large adult stem cell population [75–77]. A cut planarian fragment as small as 1/279th can regenerate into a complete worm within one to two weeks [78].

Planarian regeneration involves the coordination of several mechanisms. After injury, the wound is closed with the help of muscle contraction [79], followed by the proliferation of a mass of new cells (called the blastema) at the injury site [80] counterbalanced by an increase in cell death (apoptosis) [81]. Regeneration

completes by a re-patterning of both the old and the new tissues, producing a new worm with all the parts adjusted to the new proportions for its now smaller size [82,83]. Many experiments have shown the existence of a carefully orchestrated communication between the new and old tissues necessary for the planarian regeneration [84–88]. These signalling mechanisms include the diffusion of morphogens [89], gap junctional communication [86,90,91], bioelectrical signals [92–94] and the nervous system [95]. However, despite the discovery of all these necessary mechanisms, no existing model can explain comprehensively more than one or two observed properties of planarian regeneration [75,96].

The target morphology of the bodyplan in planaria (the head, trunk and tail regions pattern) can be altered precisely and persistently through a combination of amputations and the blockage of gap junction communication via octanol in the medium [86], as illustrated in figure 2. Gap junctions are structures that allow current and small molecule signals to pass directly from the cytosol of one cell to that of a neighbour [97]—a system of physiological communication that plays important roles in pattern formation [90]. The planarian wild-type morphology consists of a head–trunk–tail polar pattern along the anterior–posterior axis (figure 2*a*). Amputated trunk fragments in a medium with octanol undergo a change in the target morphology (figure 2*b*), resulting in the growth of a head in both anterior and posterior wounds—producing two-headed bipolar worms (figure 2*c*). These changes in the target morphology are persistent: subsequent amputations regenerate the same altered morphology (figure 2*d*,*e*). This is the case even though the pharmacological gap junction blocker that originally altered the target morphology (octanol) is washed out (as demonstrated by high performance liquid chromatography). The change in the target morphology is not mutagenic, because the octanol treatment does not change DNA [86] and its removal restores gap junctional communication very quickly [98].

These data highlight interesting new aspects of regeneration biology. First, the target morphology (the shape to which the animal regenerates upon damage) is stably altered by a treatment that perturbs real-time physiological signalling but does not impact the animal's genomic sequence. Second, this radical change of bodyplan and behaviour is stable with respect to the animal's normal mode of reproduction (splitting followed by regeneration), raising the possibility that such physiological changes might play a role during evolution [99]. Indeed, if such worms survived in the wild, then future scientists encountering the one-headed and two-headed worms in a pond might be tempted to sequence their genomes in a search for the speciation event. The failure of this strategy serves as a reminder that not all patterning information is present at the genetic level in an adult organism. One is immediately tempted to suggest epigenetics as a mechanism [100]: chromatin modification may certainly be involved; however, the key here is that it is not sufficient. The posterior-facing (tail) wound cells that are reprogrammed to build a head may indeed be epigenetically altered by temporary changes in gap junction-mediated signals, but this tissue is removed in subsequent cuts! The worm that regenerates as a two-headed animal in future rounds of regeneration is made from a fragment that initially is anatomically normal mid-trunk tissue. Thus, whatever the nature of the altered target morphology memory (epigenetic, bioelectrical or otherwise), it is *distributed* throughout the animal and not local—even trunk tissue knows that if damaged, then it needs to make a worm with two heads. We are currently working on formulating and testing global models of target morphology storage in bioelectrical networks of non-neural somatic cells.

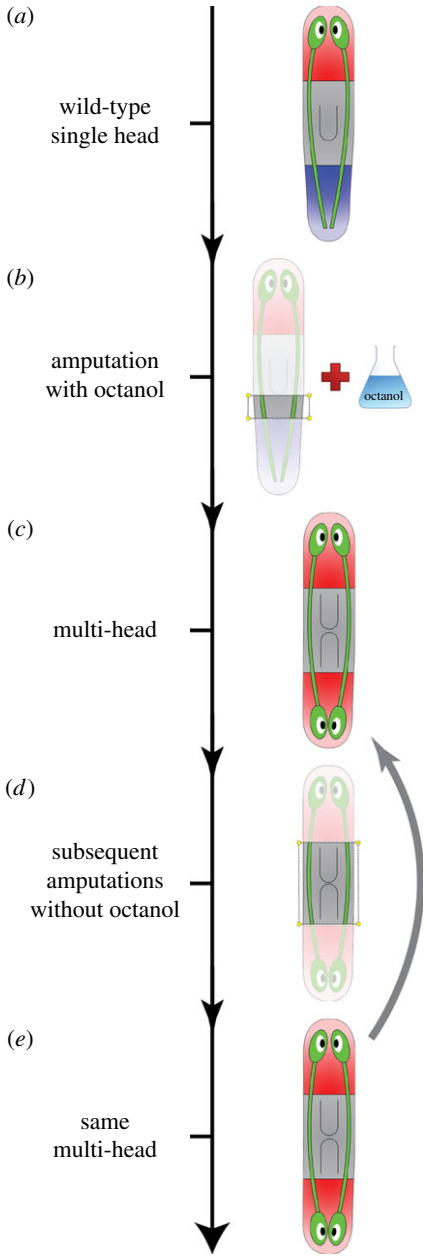

Figure 2. Planaria variable regenerative morphology. (*a*) The planarian wild-type morphology can be divided into three regions (head–trunk–tail), a pattern that is regenerated after almost any kind of amputation. (*b*) However, certain cuts under the influence of octanol in the media can produce worms with double, triple, and even quadruple heads. (*c*) A multi-headed worm not only presents an altered morphology, but also suffers a permanent alteration in the regenerative target morphology. (*d*,*e*) Subsequent cut fragments, even without the drug that induced the alteration, regenerate the same altered morphology. Worm experiment diagrams extracted from Planform [151].

2.3. Variable target morphology in fiddler crab regeneration

Adult male fiddler crabs (*Uca lactea*) possess two asymmetrical chelipeds with different size: the major chela (crusher)

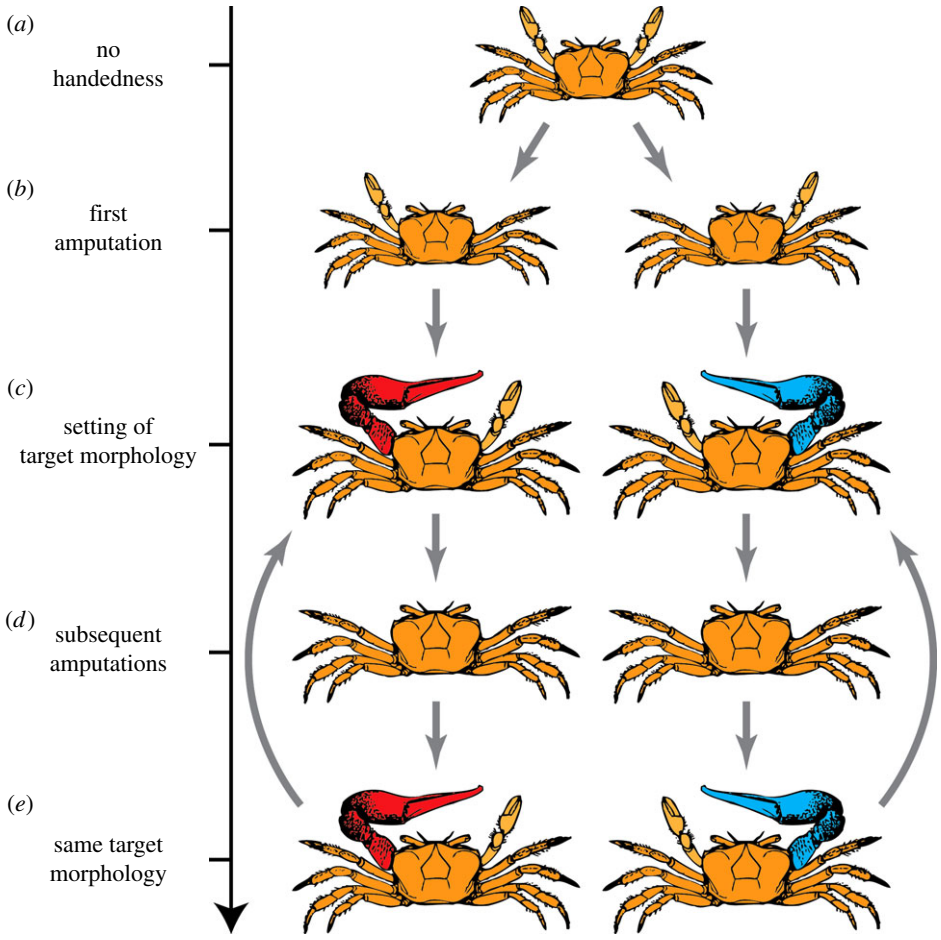

Figure 3. Fiddler crab variable regenerative morphology. (*a*) Fiddler crabs do not possess an innate handedness, developing two similar chelipeds during development. (*b*) During growth, one of their chelipeds, with equal probability, is lost. (*c*) This event establishes the location of the giant cheliped and the regenerative target morphology in the crab—left- or right-handed. (*d*,*e*) Further amputations of any or both chelipeds result in the regeneration of the same morphology, that is, the same handedness established with the first amputation.

used for aggressive and courtship displays, and the minor (cutter) used for prey capture and grooming [101,102]. Like many crustaceans, fiddler crabs can sever their own limbs (autotomy reflex), after which they can regenerate a new appendage [103]. In contrast to shrimps, lobsters and other crabs, the adult fiddler crab has a permanent handedness (left or right, with equal probability [104]), which is not genetically determined, but attained during the early years of development [104,105].

Hormones have an important role in the regulation of moulting and limb regeneration in crabs [106,107]. RNAi-mediated silencing of the genes encoding the ecdysteroid steroid hormone receptors, *EcR*/*RXR*, arrests blastema formation and inhibits the regeneration of functional limbs [108]. However, no physiological mechanism is known for the control and maintenance of the handedness in the fiddler crab, although a dynamical mathematical model has been suggested [109].

Figure 3 illustrates the acquisition and maintenance of the fiddler crab handedness, that is, the establishment of its target morphology. Crabs develop two small chelipeds of equal size (no handedness; figure 3*a*), but, during this early stage, a cheliped is naturally lost (both sides with equal probability [104]; figure 3*b*). After losing a cheliped, the remaining cheliped then develops into the giant size, whereas the lost cheliped is regenerated to the original small size (figure 3*c*). This first amputation of a cheliped establishes permanently the target morphology in the crab. Further amputations of the giant, the normal or both chelipeds result in the regeneration of the same target morphology (handedness) acquired during the initial amputation (figure 3*d*,*e*). This phenomenon has been observed both in the natural environment and in the laboratory through experimental amputations [104,105,110].

The acquisition of handedness in the fiddler crab not only represents another example of patterning information not encoded at the genetic level, but also reveals a new mechanism to establish the target morphology in regeneration. In contrast to the deer antler and planaria, fiddler crabs do not encode an innate target morphology. Instead, during development, the target morphology (handedness) is established according to a random event: the side in which a cheliped is lost. Once this event has occurred, any further regeneration follows this established target morphology, becoming impossible for the crab to develop a different handedness. Moreover, even after amputating both chelipeds—which implies starting with the same morphology in both right- and left-handed crabs—the same established handedness regenerates. Hence, the physical encoding of the target morphology must be located not in the giant or normal cheliped, but somewhere in the crab body. Still, no experimental procedure has been found to alter the target morphology of the crab once it has been established. Finding such manipulations would shed light on the mechanisms responsible for maintaining the target morphology in these organisms.

3. Types of morphological encodings and the inverse problem

3.1. Linear and nonlinear morphological encodings

An organism that is able to develop or regenerate a body part needs to produce growth consistent with the appropriate morphology of that body part. For example, the antler morphology, the planarian bodyplan (head–trunk–tail polarity) and fiddler crab handedness information need to be stored within the organism in order to regenerate these morphologies. We can distinguish two types of morphological encoding according to the type of function necessary to decode the encoding: linear and nonlinear encodings.

A linear encoding is based on a linear mapping between elements of the encoding and elements of the outcome. The simplest linear encodings are the one-to-one encodings (also called direct encodings). Similar to a blueprint, a one-to-one encoding is formally a bijection: every element of the encoding is paired with exactly one element of the outcome, and every element of the outcome is paired with exactly one element of the encoding. During development, many organisms follow an isometric (same scale) one-to-one encoding, which are usually referred to as *prepatterns*. For example, the early striped prepatterns in *Drosophila* represent an isometric one-to-one encoding of the future morphology of the larva: every stripe of high protein concentration in the embryo corresponds to a specific segment in the larva and vice versa. More complex linear encodings are based on linear maps between elements of the encoding and elements of the outcome: a linear transformation produces the outcome according to the encoding. There are many uses of linear encodings in engineering. For example, a very simple method for image compression is run-length encoding, where sequences of the same data value are stored as a single data value and count, instead of the original run. In this way, the line of pixels 'WWWWWBBBB' (where 'W' represents a white pixel, and 'B' a black pixel) can be encoded with the shorter string '5W4B'. Hence, a symbol in a linear encoding can correspond to several locally related symbols in the outcome.

On the other hand, a nonlinear encoding (also called indirect encoding) is based on iterative methods and interconnected components, where a specific element in the code does not correspond to a specific element in the outcome. Many developmental systems are based on nonlinear encodings, because a genetic network is a nonlinear encoding of the developing morphology. For example, any branching structure (such as vascular system, lung, kidney, liver, etc.) is not linearly encoded branch by branch with individual genes, but in a regulatory network that produces the emergent branching pattern through biochemical interactions [111]. The gene-regulatory network together with the local laws of physics and chemistry govern such dynamical emergent systems. In this way, the outcome is grown (or regenerated) according to the set of rules and interactions orchestrated by the encoding.

Figure 4 illustrates the differences between a one-to-one, a linear and a nonlinear encoding for storing an artificial branching morphology. The one-to-one encoding (figure 4*a*) consists of a blueprint (blue) of the final morphology (green); every element in the encoding corresponds to an element in the morphology and vice versa. The general

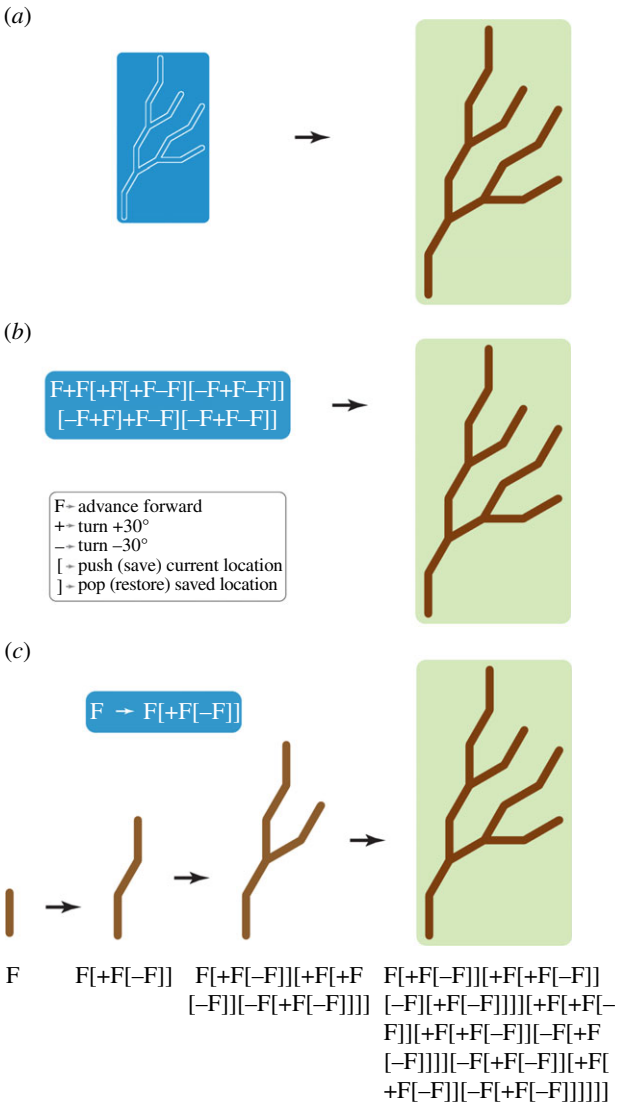

Figure 4. Different types of encodings for an artificial branching morphology. (*a*) A one-to-one encoding uses a blueprint (blue) to encode the morphology (green). (*b*) A linear encoding uses a simple algorithm (*turtle geometry* in this example) to transform a string of instructions (blue) into the morphology (green): every symbol in the string represents a movement for a 'turtle' leaving a trace. (*c*) A nonlinear encoding uses a complex algorithm (L-system rewriting grammar in this example) to create from rules (blue) the morphology (green): a final string of turtle-geometry symbols is emergently created by iteratively applying the rule three times: in each iteration, every 'F' symbol in the string is replaced by the string 'F[+F[−F]]'.

linear encoding (figure 4*b*) is based on a string of symbols (blue) that are interpreted according to *turtle geometry* [112] to produce the final morphology (green): a 'turtle' that leaves a trace moves according to the sequence of symbols read in the string. The symbol 'F' advances the turtle in a straight line (creating a segment in the morphology), the symbols '+' and '−' increase or decrease respectively the angle that the turtle is facing, and the brackets create a turtle subpath from the current position. In this encoding, a single symbol 'F' corresponds to all the constituting parts that form a straight segment of certain length, while other elements (such as brackets) have no direct correspondence with any particular element in the morphology. Finally, the nonlinear encoding system (figure 4*c*) adds an extra layer of complexity: a parallel rewriting grammar (L-system, much used for modelling biological development [113,114])

generates the string for the turtle geometry. The encoding is a single short rule (blue), which is applied iteratively, replacing the left-part of the rule (the symbol 'F') with the right-part of the rule (the string 'F[+F[−F]]'). Starting with the string 'F', the rule is applied three times to obtain a final string, which is used by the turtle geometry to generate the final morphology (green). Note that, owing to the iterative process, there is no specific relation between an element of the encoding and an element of the final morphology: a specific 'F' symbol in the rule does not represent a specific segment in the morphology.

One-to-one, linear and nonlinear encodings differ in many properties. Nonlinear encodings can achieve great efficiency when encoding repetitive patterns (with the expense of higher computational time): figure 4*c* shows how a very short rule encodes a complex branching pattern (which can continue to grow in size without needing a longer rule). By contrast, in a one-to-one encoding (figure 4*a*), repeated parts in the outcome are encoded with repeated parts in the encoding, making the encoding spatially inefficient. A linear encoding (figure 4*b*) represents a balance between spatial efficiency and computational time. In addition, nonlinear encodings have valuable properties in an evolutionary context, facilitating the evolutionary emergence of modularity, scalability, adaptability, novelty and diversity with respect to a nonlinear encoding [115–118]—yet, the combination of nonlinear and linear encodings can outperform any of the two alone [119]. However, while a linear encoding for a given morphology is very easy to produce, it represents a very difficult problem for a nonlinear encoding. This characteristic can be formalized as an inverse problem.

3.2. Forward and inverse problems

The main goal of developmental biology is to explain and predict the shape that will result from a given state of an egg or embryo. The main goal of regenerative medicine and synthetic bioengineering is to learn to provide perturbations to change the course of the complex patterning system to result in desired changes in morphology (e.g. to induce stem cell derivatives to grow an eye or limb). In order to facilitate a mathematical study, the processes of biological development and variable regeneration can be considered a forward and an inverse problem, respectively. In general, a developmental or regenerative process can be represented with the following mathematical expression: $G(m) = d$, where G is the operator representing the invariable physical mechanisms that transform the parameters m, the code (including environmental factors) for the target morphology, into the output d, the developed or regenerated morphology. Using this abstraction, we can now formalize the forward and inverse problems.

The forward problem consists of finding the output d that is produced from certain given operator G and parameters m. From this definition, it is clear that biological development solves a forward problem: the morphology of the developed organism (output d) is obtained from the invariable physical mechanisms (operator G) combined with the genetic code and epigenetic information stored in the zygote (parameters m).

By contrast, an inverse problem consists of finding the parameters m that produce a given output d with an operator G. The variable target morphology in regeneration discussed in the previous section is an example of an inverse problem: in order to specifically alter the regenerative target morphology, it is necessary to find a new code (parameters m) that produces such new morphology (output d), using the invariable physical mechanisms (operator G). For example, an inverse problem would be to create a genome that would produce a starfish-shaped creature with an elephant-like foot below and vertebrate eyes at the tips of each arm.

It is worth noting that the inverse problem has been studied in many scientific fields using different terminologies [120]. In computer science, G may be called an algorithm, a program, a procedure or the rules of a machine; m may be called the input, the arguments or the input variables; and d is usually called the output. An inverse problem in computer science consists of finding the input that produces a specific output for a given algorithm. In mathematics, G may be called a function or an equation; m may be called the input or the arguments; and d may be called the output or the value returned by a function. An inverse problem in mathematics consists of finding the argument for a specific function to return a given value. In physics, G may be called a model or a formula; m may be called the parameters, the independent variables or the input signal; and d may be called the result, the dependent variables or the output signal. An inverse problem in physics consists of finding the parameters that produce a specific result in a given model.

The organisms with a variable target morphology presented in the previous sections solve an inverse problem. When a developing antler is injured, its morphology is altered with a new royal precisely at the site of the injury. Furthermore, the encoding of the antler morphology is also altered to produce this new morphology in the following regenerative cycles (figure 1). Creating this new code for the new antler morphology is equivalent to solving an inverse problem, because the local code and rules governing cell behaviour have to be altered in precisely the right way to result in this new large-scale shape. Likewise, a temporary inhibition of gap junctional communication in planaria permanently alters the encoding of the target morphology, producing two-headed animals after each cut (figure 2). Again, creating the new encoding for the altered target morphology requires solving an inverse problem: what different rules will trunk cells follow if, in the future, they are surgically isolated and called upon to build an entire worm, which has to be two-headed? Finally, in the case of the fiddler crab, the encoding of the target morphology is initially created after losing a cheliped (figure 3), also solving an inverse problem of morphogenesis.

3.3. The forward problem is easy with linear and nonlinear encodings

The forward problem, obtaining the output given the operator and parameters, is easy to solve with both linear and nonlinear encodings. In a linear encoding (including one-to-one encodings), a simple algorithm applied to the encoding transforms it into the output (figure 4*a*,*b*). In the case of a nonlinear encoding, the output is growth applying the operator to the parameters. Starting with a simple state (the zygote), a nonlinear encoding orchestrates the growth of the resultant morphology (figure 4*c*). The forward problem, therefore, is easy for both types of encodings; however, we will find important differences in the case of the inverse problem.

3.4. The inverse problem is easy with linear encodings, but hard with nonlinear encodings

Solving the inverse problem—finding the specific code that grows a given output—is straightforward with a linear encoding. Because all linear functions are invertible, we can apply the inverse function of a particular encoding to any given output to obtain its corresponding code. In the case of one-to-one encodings, it is trivial to create a blueprint from an existing building: for every element in the building, the corresponding symbol is added to the blueprint. Likewise, it is easy to know how to add another room onto such a building: draw (or copy) such a room onto the plan, precisely where it is to go, and the apparatus that interprets the plan will implement the change. The inverse problem with linear encodings such as the run-length is equally easy: the product 'WWWWWBBBB' can be easily transformed into the code '5W4B'. Indeed, the inverse problem using a linear encoding is a well-posed problem, and efficient analytical solutions can solve it. Thus, with linear morphological encodings, biologists are freed from a limit imposed by nonlinear mathematics—their task reduces to finding the mechanisms by which pattern and form are encoded in properties of tissue and by which cells interact with this information to guide their local activity.

By contrast, solving the inverse problem is very hard in the case of a nonlinear encoding. The algorithm that transforms a code into a product cannot be applied in reverse with a nonlinear encoding, because there is no reversible relation in general between output and code. For example, it is very hard to create a genome (nonlinear encoding) that produces a given morphology, because there is no linear mapping between an element of the morphology and an element of the genome. The inverse problem using a nonlinear encoding is not a well-posed problem, because there is no analytical solution to find the inverse of any nonlinear function.

Figure 5 illustrates the key difference between the linear and nonlinear encodings that makes solving the inverse problem easy or hard, respectively. With a linear encoding, a small change in the code produces a local small change in the output; but, with a nonlinear encoding, a small change in the code can produce a large change in the output. To illustrate this important difference, we computed all the possible morphologies that result from inserting a single short substring '[+F]' or '[−F]' (which by itself encodes a single segment) in all possible locations of the linear and nonlinear codes (strings) presented in figure 4. Figure 5*a* shows the resultant morphologies in the case of the linear encoding: inserting either of the substrings results in the addition of a single new tine exactly at the location of the insertion. By contrast, figure 5*b* shows the resultant morphologies in the case of the nonlinear encoding: inserting either of the simple substrings causes the development of many new branches with no direct relation between the location of the insertion and the location of the change. The large and delocalized change in the output is due to the recursive process characteristic of nonlinear encodings and the lack of a reversible mapping between encoding and product. It is clear now why a linear encoding can solve the inverse problem easily: to encode a new morphology with an extra tine, only a new short substring in the corresponding location of the new tine is necessary. Furthermore, with a linear encoding, all possible morphologies with an extra tine can be obtained by inserting the new substring in the corresponding place. By contrast, with a nonlinear encoding, it is not possible to obtain all possible morphologies in general, and there is no clear way to change the code to produce a given output.

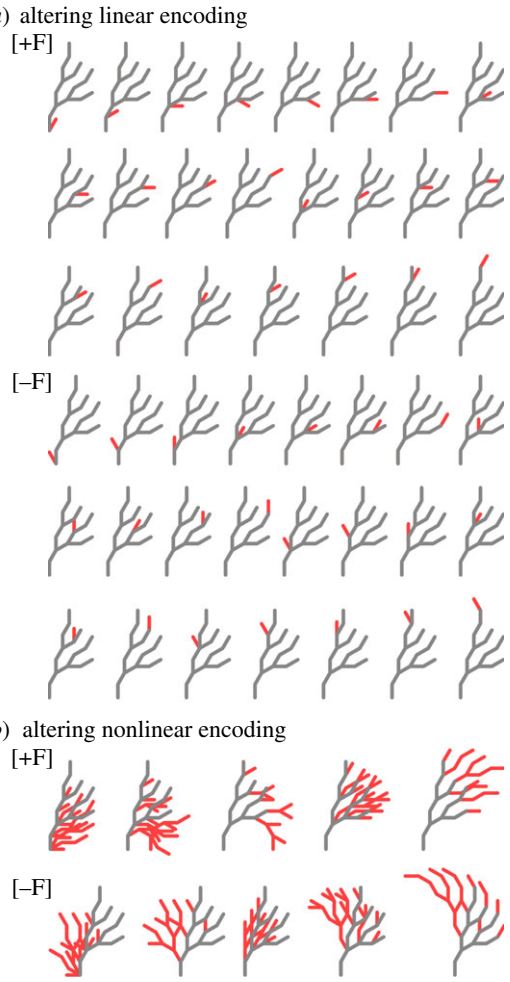

Figure 5. Slightly altering the code causes a small local change in a linear-encoded morphology, but a large global change in a nonlinear-encoded morphology. (*a*) In a linear encoding, a single substring insertion ('[+F]' or '[−F]') changes the morphology locally, adding an extra tine (red) to the original morphology (grey). Any possible morphology with an extra tine can be generated with a single simple substring insertion in the linear encoding. (*b*) In a nonlinear encoding, the effect of a substring insertion is amplified due the emergent nature of the encoding, producing a generalized large change in the generated morphologies. Only a limited set of globally changed morphologies can be generated with a single simple substring insertion in the nonlinear encoding.

The inverse problem with nonlinear encodings is pervasive in many scientific fields, and there is not a simple method to solve it in general. In the computer science field, it was formally demonstrated to be impossible to build a program (a Turing machine, which can be considered as a nonlinear encoding) to solve the general inverse problem in a finite time [121]. In a mathematical sense, inverse problems are from the class of problems where the input is not a continuous map to the output [29]. There is no general analytical solution for finding the rules that indirectly generate a given output [122]; stochastic and heuristic search methods are the usual approaches to find approximate solutions for complex inverse problems [120,123–127]. While the inverse problem of producing genomes for morphologies adapted to their environments is solved by nature via the stochastic process of evolution, such

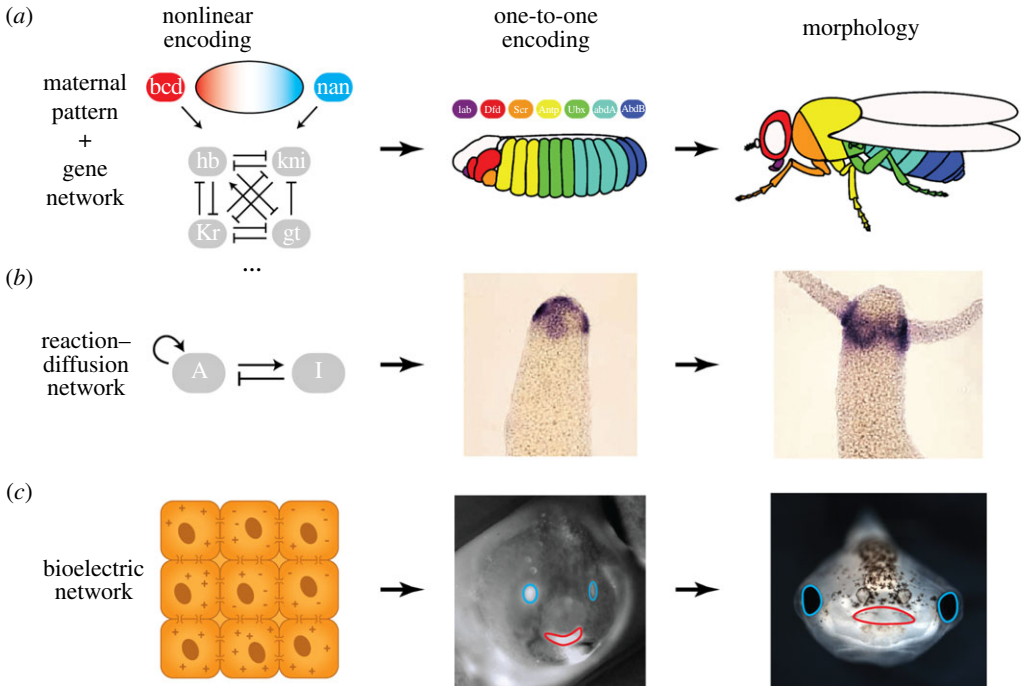

Figure 6. Many organisms develop according to a nonlinear encoding producing a one-to-one linear encoding of the final morphology. (*a*) In *Drosophila*, the maternal gene factors and a complex gene network including gap, pair-rule, and segment polarity genes form together a nonlinear encoding of the homeotic gene expression pattern that is produced in the larva embryo. This expression pattern is a blueprint, a one-to-one encoding, of the final morphology of the fly: each part of the fly morphology is determined by the expression of a homeotic selector gene. (*b*) In hydra, a reaction–diffusion mechanism (nonlinear encoding) produces a concentration prepattern (one-to-one encoding) of the *HyAlx* gene, which establishes the location where the hydra tentacles will grow. (*c*) In *Xenopus*, a bioelectric network (nonlinear encoding) produces an electrical prepattern (one-to-one encoding) of the tadpole face morphology: ectoderm regions with hyperpolarized cells (brighter) establish the developmental location of the eyes (blue marks) and mouth (red mark). Embryo and adult *Drosophila* cartoons adapted from [128]. Hydra pictures adapted from [129]. Electric frog embryo picture adapted from [130].

strategies are not good candidates for biological mechanisms of regenerative shape change because they operate far too slowly to allow real-time morphogenesis. Instead, we propose the existence of linear encodings to guide regeneration, which explains the variable target morphology showed in the model organisms presented above.

4. Linear encodings in development and regeneration

4.1. Two-step nonlinear–linear encodings in development

Many organisms follow a two-step process during development, combining a nonlinear with a one-to-one linear encoding. Figure 6 shows three examples of this two-step mechanism. During *Drosophila* development (figure 6*a*), the maternal effect genes (such as bicoid and nanos) and a complex genetic network (including gap, pair-rule and segment polarity genes) together represent a nonlinear encoding of the patterns of the homeotic gene expressions, which emerge from the genetic interaction, diffusion and reaction of such gene products [33,131–136]. However, the homeotic gene expression pattern represents a one-to-one linear encoding for the subsequent morphology of the fly: the location of the expression of every gene product specifies the location of a specific fly body part. A second example can be found in hydra (figure 6*b*), where a reaction–diffusion process [137] (a nonlinear encoding) creates a characteristic expression pattern of the *HyAlx* gene [129]—long-range pattern emerges from the chemistry of local rules. Then, this pattern serves as a one-to-one encoding that establishes a blueprint with the locations where the hydra tentacles will grow. Another example can be found in *Xenopus* development (figure 6*c*), where the bioelectric networks created by ion channels and gap junctions (nonlinear encoding) produce patterns of cells with different resting potentials [130]. These membrane voltage patterns (one-to-one linear encoding) establish the morphology of the tadpole face (figure 6*c*), including the location of the eyes (blue areas in the figure) and mouth (red area).

Similar to the artificial experiments showed in figure 5, experimentally altering the nonlinear or linear encodings in these biological systems results in global or localized changes in the morphology, respectively. In *Drosophila*, landmark experiments showed how mutations in the gap genes (nonlinear encoding) globally affect the development of many regions in the larva [131]. By contrast, mutations in the homeotic genes producing the one-to-one linear encoding pattern result in localized changes in the developed fly, such as transforming the morphological identity of one specific segment into another [33,138]. In hydra, altering the genetic and reaction–diffusion chemical networks (nonlinear encoding) can produce global changes in morphology, such as phenotypes with ectopic tentacles all over the body [139,140]. Similarly, alterations of the membrane voltage regulation of cells (nonlinear encoding) during *Xenopus* development cause global malformations in craniofacial morphogenesis [130]. Remarkably, altering the membrane voltage of a small group of non-eye cells anywhere in the body, that is, directly manipulating the membrane voltage field (one-to-one linear encoding), induces the development of

tadpoles with whole ectopic eyes [141]. Therefore, as a general guideline, localized small changes are obtained when altering a one-to-one linear encoding, but global changes are induced when altering a nonlinear encoding. Indeed, finding the necessary changes in a nonlinear encoding to produce a specific small change requires solving the inverse problem, which, as we have illustrated, is very hard in general.

4.2. A linear encoding can explain the variable target morphology in regeneration

Deer antlers, planaria and fiddler crabs can alter their encoded target morphology in a precise and lasting manner, for which an inverse problem needs to be solved by the cells that must rebuild each structure. In order to change the target morphology, they must know the local actions that will produce the new morphology. However, we have seen that solving the inverse problem with a nonlinear encoding is very hard. We propose, therefore, the existence of a linear encoding of the target morphology in these regenerative organisms, which can explain the variability of their regenerative morphologies. More broadly, we argue for a greater consideration of linear target morphology models in developmental and synthetic biology: the community must consider and test not only emergent nonlinear models popular in systems biology and complexity science, but also models that postulate an explicit encoding of target shape.

As in the developmental systems of *Drosophila*, hydra and *Xenopus* shown in figure 6, deer antlers and planaria may use a two-step process during development, combining an initial nonlinear with a lasting linear encoding. Figure 7*a* illustrates this mechanism. A transcriptional network encoded in the genome represents a nonlinear encoding, which produces a linear encoding, represented by a list of sequential instructions (antler) or a blueprint (planaria) of the target morphology to develop. This linear encoding can readily orchestrate the regeneration of cast antlers, or amputated planarian body parts. Importantly, this mechanism can explain the variable target morphology present in these organisms. Figure 7*b* illustrates how injuries or drugs can locally alter the linear encoding, which will produce a local modification in the regenerated morphology. For example, an injury during the development of a tine in the deer antler may alter the stored linear encoding exactly at the location where that tine is encoded, producing a local modification of the morphology of the tine in the following regeneration cycles. Similarly, the temporary modification of gap junction-mediated signals among distant cells in amputated planaria may change the linear code to one in which transverse amputation generates bipolar two-headed animals. This new code defines the target morphology of any further amputations, explaining the regeneration of the same altered morphology even in the absence of any octanol in subsequent rounds of repair. In the case of fiddler crabs, the code for their handedness (left or right location of the giant cheliped) is due to the random event of losing a cheliped. The first amputated cheliped determines the linear code of its target morphology, and any further amputation restores the target morphology according to this linear code.

The combination of nonlinear and linear encodings can also explain the eventual recovery in deer of the original morphology after its alteration: because the nonlinear encoding is never changed during the injury experiments, it can recreate the original linear encoding. After a few regeneration cycles, antlers altered owing to an injury tend to slowly recover their original morphology—the genetically stored nonlinear encoding may be restoring the original linear encoding, a process that takes several cycles (figure 1*b*). Similarly, the two-step encoding of the target morphology can explain why certain fragments from two-headed worms regenerate again the wild-type morphology [86]. These fragments may have completely lost the linear encoding of the target morphology, and, instead, a re-development from the nonlinear encoding stored in the genome results in the original linear encoding and the regeneration of the wild-type morphology.

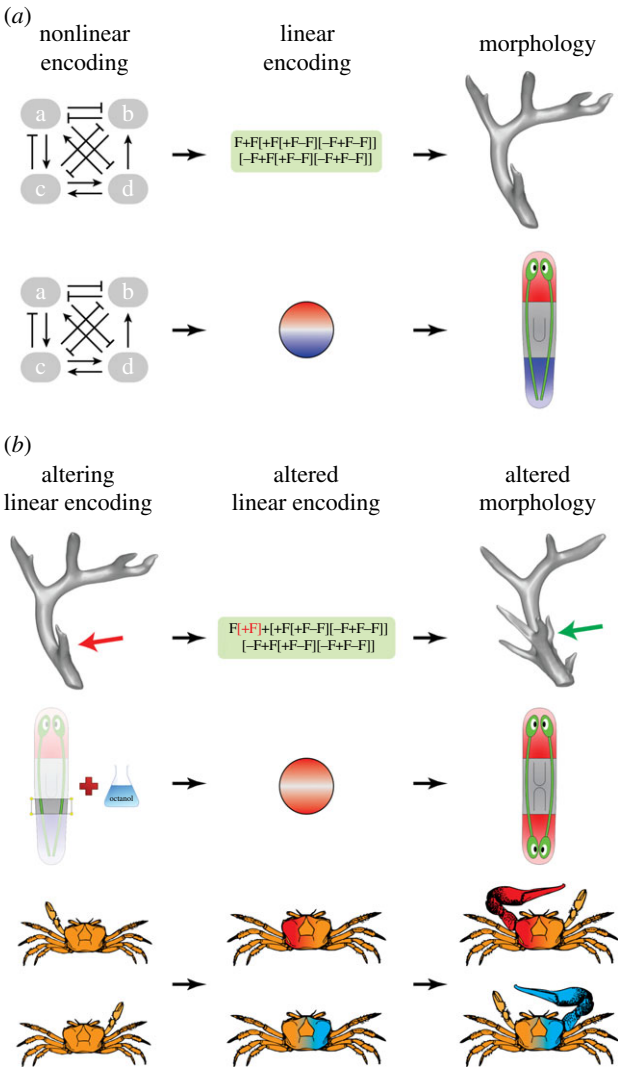

Figure 7. A linear encoding can explain the variable target morphology in regeneration. (*a*) During development, a nonlinear encoding mechanism (such as a genetic network) produces a linear encoding that establishes the target morphology of development and regeneration (antler structure or planaria bodyplan). (*b*) Injuries or drugs can alter locally the linear encoding of the target morphology, which will cause a corresponding local change in the regenerated morphology. An injury in an antler tine (red arrow) can alter the encoding of that tine in the code, which will cause the regeneration of the antler with a modified morphology (a royal, green arrow) exactly at the location of the tine—a phenomenon hardly possible with a nonlinear encoding. Similarly in planaria, injuries combined with octanol cause the modification of the linear encoding of the body pattern (represented by the patterned circle in the figure), causing the subsequent regeneration of the same altered morphology. In the fiddler crab, the first amputation of a cheliped establishes a linear encoding (represented with a coloured body in the figure) of the crab handedness, which dictates the location of the giant cheliped in subsequent amputations.

Table 2. Types of encodings for the target morphology.

encoding type	code	decoding process	forward problem	inverse problem
nonlinear	genetic networks, recursive rules	recursive mechanism	easy	hard
linear	simple rules	forward mechanism	easy	easy
one-to-one	prepattern, blueprint	replacing map, scaling	easy	easy

Importantly, not all linear encodings need to be isometric (like a prepattern), but they can exist on a different scale from the morphologies that they encode. In the same way that an architect blueprint or compressed image has a smaller size than the building it represents, a linear encoding can be physically smaller than the morphology it encodes. In particular, the linear encoding in deer antlers, planaria and fiddler crabs cannot be a one-to-one prepattern as illustrated in *Drosophila* development (figure 6*a*). The linear encoding of the deer antlers must reside in a smaller scale outside of the antlers themselves, because they are cast every year and grown anew from the pedicles. In wild-type planaria, amputating a piece of the worm does not alter its target morphology, meaning that it should be encoded redundantly over their bodies in a smaller scale. Similarly, chelipeds with the correct size are developed even from crabs with both chelipeds amputated. A difference in scale between the encoding and the morphology implies the existence of a method to generate the scaled-up morphology form the linear encoding. Examples of such methods include means-end algorithms that specifically consult the linear encoding to perform the actions necessary to reach the right morphology from the current state. Notably, these algorithms predict the extraordinary capacity during embryonic development to fix perturbed morphologies [38].

Many biological mechanisms can store a linear encoding of the target morphology. The trophic memory in antlers has been suggested to be physically located in the deer nervous system, possibly in the brain [65,66]. Although a neural network is powerful enough to store nonlinear encodings, it is also possible for it to store any less complex encodings, such as the linear encodings that we propose here. Moreover, because the antlers are innervated, the trauma information can be sent through the neurons located in the antlers, which can precisely alter the encoded target morphology (solving the inverse problem), but only if this encoding is linear. Interestingly, recent data using morphometrics and laser ablation in the *Xenopus* larval tail model revealed that the central nervous system far away from the wound site seems to carry instructive information for shape of the regenerated appendage [142,143]. Other ways of storing morphological information with a linear encoding include chemical maps maintained by feedback loops (such as the transcriptional memory of *Hox* genes [144]), physical structures (such as nerve cords) or electrophysiological mechanisms based on gap junctions (as suggested for cardiac memory [145,146]).

5. Conclusion

Deer, planaria and fiddler crabs possess the capacity to alter the target morphology achieved through regeneration, a phenomenon that requires altering the individual behaviours of thousands or millions of cells to achieve a large-scale anatomical goal state. If we accept the common view that living systems are fundamentally computational in nature [147–149], then these organisms necessarily use a linear encoding for their target morphology because they are able to alter it precisely in the location of specific injuries and, hence, solve an inverse problem. We have argued in this paper that a combination of a nonlinear and a linear encoding can explain the variable target morphology during regeneration. While a nonlinear encoding can facilitate the evolution of modularity and diversity, a linear encoding may be an efficient mechanism to facilitate regeneration.

Nonlinear and linear encodings differ with respect to exactly what is specified (encoded) in the physical medium, and how direct (how much decoding) needs to take place to derive the target morphology. Nonlinear encodings specify recursive rules for cell or molecule behaviour: the morphology emerges as a result of applying the rules. Examples of nonlinear encodings include gene-regulatory networks specifying cell interaction rules and cellular automata such as the game of life. On the other hand, linear encodings specify simple rules (a linear transformation) that need to be applied to the code to obtain the final morphology. Prepatterns are the simplest type of linear encodings: a one-to-one map exists between the code and the target morphology. There are many examples of prepatterns in developmental model organisms, such as the Hox gene gradients in *Drosophila* that directly specify axial identity. However, not all linear encodings are prepatterns; other linear encodings can bear little or no direct relationship to the final shape. Examples of this more complex type of linear encoding include simple algorithms for compressing images and turtle graphics methods to generate complex shapes. The differences between these encodings are summarized in table 2.

The fundamental difference between nonlinear and linear encoding models suggests the need to expand the current approach in regenerative biology from an exclusive focus on genetic networks and pathways (nonlinear encodings), to take into account models based on spatial encodings of morphological information. Importantly, the linear encoding of the target morphology's representation in tissue properties can be very straightforward (such as Hox gene prepatterns that specify underlying tissue fate), or they can be encoded in a more complex manner (linear transformations). For example, a neural network (or a bioelectrical network of non-neural cells [69,150]) could store information that guides growth towards a specifically remembered shape, but the information is not stored in a simple 'image' of the final product but in the distribution of node activation strengths. Either type of encoding can function as target end states of cybernetic goal-seeking mechanisms, such as algorithms that form a shape by comparing the current state with a target state—a novel approach to model a range of regenerative phenomena as discussed above.

Thus, our proposal differs from existing models of chemical prepattern in these two critical ways: (i) we propose that

target morphology can be explicitly encoded in cell properties far more complex than gradients and prepatterns corresponding to spatially overlying tissue fate and (ii) this information, owing to its linear nature, could be still explicitly 'read' by processes seeking to repair and remodel shape, and 'written' by processes that alter the pattern to which future growth should conform.

Indeed, models based on a linear encoding of the target morphology have unique testable implications that are not predicted by any existing emergent genetic model. A linear encoding predicts the capability to experimentally produce precise and lasting morphological alterations *during* the lifetime of a single organism, as we have discussed for deer antlers, planaria and fiddler crabs. Indeed, planaria are some of the most plastic model organisms in regenerative biology, with more than 250 known experimental phenotypes, as recorded in the planarian phenotype database Planform [151]. By contrast, nonlinear encoding models can account only for a much reduced phenotype landscape (those phenotypes that can be produced with a specific set of rules) [119]. Moreover, a linear encoding mechanism can readily explain why cancerous cells grafted in developing embryos or regenerating organs stop their neoplastic behaviour and become integrated as normal tissue [152–156]. A linear encoding mechanism during development and regeneration directly contains spatial information of the target morphology, which can reprogram the cancerous cells according to their location to become part of the encoded target morphology. Linear encoding models thus suggest alternative approaches to cancer normalization (in line with the views of cancer as a problem of tissue organization [157,158]) focused on activating cellular responses to fields of non-local patterning information [21,34,159,160].

In addition, the inherent plasticity of linear encodings is of exceptional importance in regenerative biomedicine. Finding the changes in a nonlinear encoding necessary to emergently restore a desired morphology (solving the inverse problem) is a very complex task and currently regarded as a long-term goal in regenerative medicine. By contrast, properly altering a linear encoding to produce a specific morphology is a much easier task. This was demonstrated with the electric map during *Xenopus* development: an ectopic eye can be experimentally induced in any desired location by changing the transmembrane voltage levels (linear encoding) present in the embryo precisely in that location [141]. Cells were coaxed to implement a complex organ without the need for the experimenter to micromanage the progress. Thus, the discovery of morphological linear encodings would pave the way for novel medical procedures to regenerate amputated body parts long before we had the capability of building such a complex structure directly or of altering gene regulatory networks to make the needed change and no more.

Finally, this new perspective on regeneration can also benefit the engineering and computational fields, inspiring novel mechanisms for resilient self-assembly robotics [161–163] and novel heuristics for evolutionary computation algorithms based on hybrid nonlinear–linear encodings [119].

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