Physical forces in regeneration of cells and tissues

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I. Introduction

The ability to regenerate structures after injury is one of the hallmarks of life (Koshland 2002). Many animals are able to re-build missing structures, such as limbs, or even regenerate entirely new bodies from small fragments of an organism. In humans, regeneration is less extensive than in invertebrates or fish, but even then, regeneration is possible. For example, the liver can grow back to its normal size after up to 90% of it is removed, and the skin is usually restored to a condition equivalent to that before a cut. Young children can even regenerate their fingertips. On the other hand, human are not very good at regenerating spinal cord axons.

There are three major reasons to study regeneration: First, regeneration is a fundamental aspect of living things, such that we cannot say we understand how life works if we don't understand how living things regenerate. Second, learning how regeneration works may point the way to therapeutic approaches to help injured patients regenerate and recover function. Third, regeneration provides a window into the mechanisms of development by allowing development to be induced and by allowing developmental pathways to be studied outside the normal context of the embryo. Throughout the history of biology, regeneration has been studied for all these reasons to varying extents.

Regeneration occurs at both the tissue and cellular scales

Figure 1 gives several examples of regeneration that span scales from a whole multicellular organism to sub-cellular structures. The examples shown span orders of magnitude in terms of both spatial and time scale required for regeneration.

As a result of over a century of intensive research using embryological, genetic, and molecular tools, we have a wealth of information about such regenerative processes at the tissue, organ, and whole-animal scales (Tanaka 2011; Maienschein 2022). But this is not to say that we know how regeneration works. Despite having a long list of genes involved, many questions remain about the actual morphogenetic processes - how do newly differentiated replacement cells "know" how to build structures of the correct size and shape, in the right position and orientation? As in embryonic development, pattern formation and morphogenesis in regeneration involves an interplay of molecular cues and physical processes.

Regeneration also takes place at the level of individual cells. Cells are fragile structures that can easily be injured, either during pathology, predation, or even due to forces associated with normal organismal function. Muscle cells, for example, become injured during normal exercise. For free-living single-celled organisms, such as those swimming in a pond, the world is a dangerous place full of sudden environmental changes in pH or salinity, not to mention predatory organisms equipped with sharp mouthparts capable of chopping off pieces of a prey cell. When cells become injured such that some of their components are lost, they are often able to regenerate those missing components. Cell regeneration can entail both re-formation of missing organelles, as well as re-establishment of the correct overall shape and polarity. As with tissue or organ-level regeneration, cellular regeneration provides a way to study the development of cell geometry (Shulman 1999; Kirschner 2000; Harold 2005; Marshall 2016), by allowing morphogenesis to be triggered at a time of the investigator's choosing.

Common themes in regeneration across scales

Many parallels exist between regeneration at the tissue and cellular scales. In both cases, the same three processes are required: (1) wound closure to stop further loss of material; (2) detecting that damage or loss has occurred in order to trigger subsequent regeneration pathways which may involve formation of new material or respecification of existing material; and (3) re-establishing correct overall patterning. The major difference in the two scales is that at the tissue level, much of regeneration involves migration, proliferation, and differentiation of stem cells to replace the cells lost during damage, whereas at the cellular level, there is only one cell to start with and so other explanations need to be sought. In both cases, molecular pathways and physical forces both play a role and are tightly coupled, but we imagine that at the cellular level, the latter may be more important just because pattern formation cannot be explained in terms of cell differentiation. We recognize, however, that even at the tissue scale, much of regeneration is ultimately physical - changes in geometry and size - such that a strict focus on genetic mechanisms is unlikely to be sufficient to understand how regeneration works.

Wound healing versus regeneration

Wound healing and regeneration are sometimes treated as separate processes, but in reality they are highly intertwined. Wound responses are often necessary to trigger the initiation of regeneration (Polezhayev 1946; Newman 1974; MacWilliams 1983). Here we will consider both wound healing, and the subsequent rebuilding of lost structures, together under the more general heading of regeneration.

Morphallaxis versus epimorphosis

Regeneration of part of a multicellular organism requires the availability of the appropriate cell types, which can happen in two ways. At one extreme, the cells used to rebuild a structure are ones that were already present prior to the loss of the structure, and they simple rearrange their positions as necessary. This extreme case is sometimes known as "morphallaxis". At the other extreme, the necessary cells are obtained by differentiation of undifferentiated precursor cells, either stem cells (neoblasts) existing prior to loss of the structure, or cells that de-differentiate as part of a "blastema" forming neat the wound. This extreme alternative is referred to as "epimorphosis". The degree to which these two processes are involved in regeneration is likely dependent on the organism and tissue (Maienschein 2022).

The concepts of morphallaxis and epimorphosis also apply at the sub-cellular level. If an organelle or other structure needs to be re-built, it is an open question whether the necessary molecular components are synthesized de novo (equivalent of epimorphosis) or obtained by disassembling existing structures and re-arranging the molecular building blocks into a new structure. A clear example of the latter is regeneration of flagella in Chlamydomonas. The Chlamydomonas cell has two flagella, which are virtually identical to cilia in animal cells. If one flagellum is detached, it immediately begins to re-grow, which requires a continuous supply of tubulin. At least some of this tubulin is obtained by disassembly of the other flagellum, which shortens as a result. It has been directly demonstrated that the tubulin obtained from flagellar disassembly can be used for flagellar assembly (Coyne 1970; Lefebvre 1978).

Goal of this review

The vast majority of what we know about regeneration concerns the molecular pathways that control the process. Compared with this molecular understanding, the physics of regeneration remains far less understood. One reason is that the methods for studying forces and mechanics in vivo are less scalable and harder to employ than current "omics" methods. A second reason is that, due to the more extensive knowledge at the molecular/genetic level, conceptual thinking about regeneration has tended to focus on questions of gene expression and its regulation, rather than about the mechanics that must accompany regeneration itself. This review will attempt to summarize what little is known about physical forces in regeneration, not with the goal of providing a comprehensive and complete explanation of regeneration in physical terms, but of hopefully stimulating interest and further investigation into this area. Due to space constraints we focus on animals and on two specific single-celled organisms, Chlamydomonas and Stentor.

II. Physical forces in wound healing

Wound healing is a basic attribute of all living systems. The term "wound" often refers to a hole in a sheet, such as an epithelium or cell membrane, which can occur by a puncture through the sheet. In the context of regeneration, wounds often reflect an opening in an epithelial sheet over the stump left behind when an appendage is severed. Wound healing refers to the closure of the sheet, to restore integrity to the tissue or cell and prevent further loss of material.

Wound healing at tissue scale

Among the most well studied systems is wound healing of the epithelium. Wounds in epithelial sheets are generally closed by two mechanisms (**Figure 2A**): crawling of cells into the hole, or formation of an actomyosin "purse string" around the rim of the hole which then draws it close (Martin and Lewis 1992; Jacinto 2001; Wood 2002; Klarlund 2012). The actomyosin purse string is formed from actin and myosin assemblies within the cells on the rim of the wound, which are linked together to form a connected contractile unit (Bement 1993; Brock 1996). Traction force microscopy measurements have found that the purse string can generate radial forces in the range of tens of nanonewtons up to micronewtons (Brugues 2014; Vedula 2015). But for the wound to close, it is not enough to pull on the cell sheet, it is also necessary for the cells around the wound to be able to re-arrange themselves, such that the ability to remodel cell-cell junctions, known as tissue fluidity, plays a key role in allowing wound closure (Tetley 2019; Mosaffa 2020). The actomyosin purse string is often thought of as a structure that generates tension within itself to draw the wound close, but careful measurements have shown that the purse string also transmits forces to the underlying substrate through focal contacts, and the combination of these two types of forces is necessary to account for the dynamics of wound closure (Brugues 2014). In some cases, wounding of an epithelium results in extrusion of the damaged cell at the wound site, and it has been shown that actin-myosin forces generated within the wounded cells themselves can contribute forces to help speed up wound closure (Fernandez-Gonzalez 2013).

In highly regenerative animals such as planarian and hydra, dissected tissues are found to contract or fold around the wound edges to reduce the wound size. In planarian, this contraction is driven by a muscle layer under the epidermis (Chandebois 1979), although the detailed mechanism of the contraction is not fully understood. In hydra, the contraction (or folding) is driven by a contractile actomyosin cytoskeleton aligned over supracellular scales: the ectoderm of hydra contains actomyosin bundles aligned longitudinal to the body axis, and the endoderm contains actomyosin bundles aligned circularly (Livshits 2017). An excised flat piece of epithelial tissue is found to bend (with ectoderm on the outside) and close into a spheroid, thereby reducing the wound size (Livshits 2017). Recently, an active-laminated-plate model is used to predict this bending by accounting for the anisotropy in contractility and elasticity of the tissue (Su 2022).

Wound healing at cellular scale

There is increasing recognition that single cells have a wound response. Single-cell wound healing is found in fungi, amoebae, budding yeast, and also cells of Metazoa (McNeil 2003; Bement 2007; Schapire 2009; Nakamura 2018). These studies have indicated that the healing of plasma membrane wounds involves active cellular processes (Moe 2015; Sonneman 2011). At least three steps are involved when the plasma membrane is disrupted (**Figure 2B**) (McNeil and Steinhardt 2003; Sonneman2011; McNeil2003b): (1) The influx of calcium ions and/or oxidative species through the membrane opening triggers the beginning of the wound healing process, (2) trafficking of internal membranes to the wound site to seal the plasma membrane, and (3) rearrangement of the cortical cytoskeleton. Multiple modes of membrane trafficking have been reported to close the hole in the plasma membrane, including, for example, membrane patching or plugging where internal compartments fuse or crosslink at the wound site (Moe 2015; Jimenez 2017). Nevertheless, a number of membrane trafficking modes (e.g., exocytosis, internalization of plasma membrane-containing wounds via invagination, externalization of plasma membrane-containing wounds) are likely to be limited to closing small wounds (Moe 2015). To assist with wound closure, other strategies involving active force generation are employed. Large cells such as Xenopus oocytes and Drosophila embryos build a contractile actin "purse-string" around the wound that appears similar to the purse string formed in tissue scale wound closure (Ebstrup 2021; Heitman 2021; Hui 2022). By active contraction, the actin ring reduces the wound size to a range where smaller-scale membrane trafficking modes could possibly complete the patching of the wound. Several mechanisms have been proposed on how exactly the actin ring contracts (Hui 2022): (1) sarcomere-like contraction, where actin filaments slide past each other due to motor processivity; (2) actin filament treadmilling, where actin is continuously assembled at the inner edge of the ring, and disassembled at the outer edge of the ring; (3) F-actin buckling, where actin filaments are mechanically deformed by myosin. Recently, it was found that Xenopus oocytes with reduced intracellular osmotic pressure and corresponding membrane tension do not form a contractile actin ring after wounding (Kato 2021). While it is known that membrane tension modulates membrane repair (Togo 2000), this result suggests that the cortical actin cytoskeleton may act as a mechanosensor to direct its own repair.

Among single celled organisms, wound-healing has been studied most extensively in the giant ciliate Stentor coeruleus (Tartar 1961; Marshall 2021). Stentor was a popular organism for studying regeneration at the single-cell level since the early 1900s, notably by Thomas Hunt Morgan and Vance Tartar, in part because it is large (up to 2 mm) and that it is possible to manually cut pieces from one cell and keep them alive long enough to graft them onto another cell. Stentor contains a highly polyploid macronucleus such that even small cell fragments can contain enough genomic copies to survive and regenerate, but the key to its utility in these cut-and-paste type experiments is its wound healing ability. Stentor can heal wounds with a diameter close to half of the cell diameter, with a healing rate of ~ 8–80 $\mu m^2/s$, faster than most other single cells reported in the literature (Zhang 2021).

Why doesn't the cytoplasm leak out uncontrollably when a Stentor cell is cut? It is well known that the cytoplasm is a complex active matter (Mogilner 2018; Heidemann 2004; Kasza 2007; Mitchison 2008). It is thus possible that the viscosity of the cytoplasm around the wound increases locally to restrict leakage, possibly due to the increased density of cytoskeletal elements. However, mechanisms driving this change in cytoplasm rheology in Stentor remain unknown.

Upon bisection in a microfluidic “guillotine” (Blauch 2017), Stentor is observed to employ unique, large-scale mechanical modes of wound response. Three distinct mechanical modes of wound healing in Stentor are found (**Figure 2C**): contraction of one area of the cell surface to fold over the wound (Zhang 2021), cytoplasm retrieval (Slabodnick 2013; Zhang 2021), and twisting or pulling (Zhang 2021). Most wounded Stentor cells utilize at least one of these mechanical modes, which suggests that the mechanical modes play an important role in Stentor wound response.

Cytoplasmic flow in wounded cells has also been reported in as other cell types such as plant cells and amoeba (Nichols 1925). The twisting mode of wound healing is remarkably similar to rotokinesis, which is required for Tetrahymena cell division (Brown 1999). Overall, large-scale mechanical behaviors, such as cellular force generation and contractility, may be of greater importance to wound healing in free-living single cells than previously thought.

III. Decision making - what cues trigger regeneration?

One of the most fundamental questions in regeneration is how does the system "know" it is time to trigger a regeneration response? Once the trigger is activated, new material can be synthesized and moved to the correct position, but how is the need for regeneration sensed in the first place? The triggering of regeneration is of particular interest with respect to the mechanisms of patterning, because it raises the possibility that organisms possess a machinery to sense or monitor their shape or pattern, in order to be able to recognize when something is amiss. Compared to mechanisms for generating patterns, mechanisms for monitoring patterns within an organism have been less investigated.

Triggering regeneration at tissue scale

The act of wounding a tissue triggers a number of intercellular signaling pathways (Petersen 2009; Cordeiro 2013; Roberts-Galbraith 2013; Gavino 2013; Reddien 2018), some of which are common in any wound, while others are specific to wounds associated with tissue loss (Wenemoser 2012), also known as the "missing tissue response" (Wenemoser 2010). But how is the loss of material sensed in the first place?

One possibility is that each tissue or organ emits a signal that inhibits its own regeneration, such that when the structure is removed, the inhibitory signal is removed, and regenerate ensues. A classic example is the regeneration of the eyestalk in crustaceans, in which a hormone produced by an endocrine gland within the eyestalk (the "X-organ") inhibits the regeneration pathway by acting on a second neuroendocrine region (the "Y-organ") within the brain (Mykles 2021), preventing it from releasing ecdysteroid hormones necessary to trigger molting, which is part of the regeneration process (**Figure 3A**). When the eyestalk is severed, the source of the inhibitory hormone is removed, thereby inducing regeneration.

But chemical signals need not be the only way that a structure, when present, might inhibit its own growth. Given the fact that many tissues are under mechanical tension (Farhadifar 2007; Fletcher 2014), one could imagine a mechanism in which the sudden loss of a structure was sensed via a sudden decrease in tension, which would then be transduced into the activation of a regeneration pathway. Within nerve tissue, it is known that mechanical tension can affect the stability and outgrowth of axons, with tension generally favoring axon outgrowth (Bray 1984; Anava 2009; Heidemann 1994; Dennerll 1989; Zheng 1991; Lamoureux 2002; Fass 2003). After outgrowth is completed, axons remain under mechanical tension (Siechen 2009). Given that tension can regulate growth, and severing of an axon would lead to a sudden and acute loss of tension, one might speculate that this loss of tension could serve as a trigger for axon regeneration . Indeed, the mechanotransducer Piezo1 is known to regulate regeneration of axons (Song 2019), and one model considered by those authors was that Piezo1 was involved in sensing the loss of tension when an axon is severed. However, it seems that the tension sensed by Piezo is not the tension present prior to cutting, but rather tension generated by the growth cone as the axon regrows (Song 2019). Overall, it seems unlikely that tension is a trigger for nerve regeneration, which instead seems to be controlled primarily by a calcium signal that starts at the site of wounding and propagates back to the cell body either as a calcium wave or by retrograde transport of downstream signaling components (Smith 2020).

In the case of epithelia, wounding in the form of a hole or opening in the epithelial sheet leads to a loss of tension and a pullback of the surrounding cells (Farhardifar 2007). The subsequent process of wound closure creates mechanical tension that can both trigger proliferation of surrounding cells and also orient their migration to the site of the wound (Yannas 2017). This mechanical stimulus can reach substantial distances away from the wound site. In a recent study, it was found that regeneration of the zebrafish tailfin after severing was accompanied by a wave of cell migration driven by local recruitment of epithelial cells to cover the wounded area (De Leon 2023). This migration wave drives proliferation of cells far from the wound site, an effect that depends on mechanical tension in the migrating cell sheet, providing a clear example of long-range mechanical signaling in regeneration. This regulation of proliferation takes place via molecular signaling pathways that respond to mechanical forces (Paci and Mao, 2021). The role of tension sensing in epithelial regrowth is thus reminiscent of its role in nerve tissue - the tension that is sensed is not the loss of tension accompanying the loss of the structure, but forces created by the attempt to rebuild the structure.

Another long-range cue is electrical potential gradients. Epithelial sheets contain ion transporters that can develop a potential difference across the sheet. When the sheet is wounded to create a hole, the potential collapses locally around the wound, creating a potential difference in the plane of the sheet. Many cell types are able to sense such a potential gradient and migrate along it, a process known as galvanotaxis (Zhao 2009; Allen 2013; Kennard 2020).

Physical forces also help ensure that the number of cells is just enough to fill the area completely, without piling up in a jumbled mess. Cells under compression reduce their proliferation rates while cells sunder tension increase their proliferation rates (Chen 1997; Nelson 2005; Gudipaty 2017).

Triggering regeneration at cellular scale

Given that cells can regenerate lost structures, the same question arises as at the tissue level - what is the signal that triggers regeneration? Here we will discuss two cases in which removal of an organelle triggers a defined transcriptional response that allows the structure to be re-built in its former location: regeneration of flagella in Chlamydomonas and regeneration of the oral apparatus in Stentor.

Triggering regeneration of Chlamydomonas flagella

In Chlamydomonas (Wemmer 2007), pH shock causes the flagella to be shed. Upon restoration of normal pH, the cell grows new flagella in roughly an hour, a process that requires induction of a complex program of gene expression (Schloss 1984; Stolc 2005). When flagella re-grow to their normal length, gene expression is shut down back to basal levels. It was proposed that calcium might leak in when flagella detach, and then trigger gene expression (Evans 1997), similar to the role of calcium in triggering axon regrowth in neurons. However, such a direct response to wounding is not necessary ,since when full-length flagella are induced to elongate or shorten, without being actually removed, expression of flagellar genes still increases or decreases, respectively (Periz 2007; Chamberlain 2008). These data suggest that some factor related to flagellar growth may play a role in generating the necessary signal. Mutants affecting protein transport into flagella regenerate slowly and show greatly reduced expression following flagellar detachment (Perlaza 2022), suggesting the process of growth might be the trigger for precursor synthesis, possibly due to depletion of an auto-regulatory repressor of transcription (**Figure 3B, C**). This model depends on the observation that transport of proteins via IFT into the flagellum is most rapid when the flagellum is short, and slows down as the flagellum reaches its steady state length (Engel 2009; Ludington 2013). Rapid import of repressor into the flagellum during the early stages of growth would trigger gene expression.

Triggering regeneration in Stentor

In Stentor, removal of the oral apparatus (OA), a ring of cilia at the anterior end of the cell, triggers a complex transcriptional program organized as a cascade (Sood 2022). A currently open question is what triggers this transcriptional program. One possibility, analogous to the crustacean eyestalk model, is that the OA continually transmits an inhibitory signal, such that when the OA is removed, the inhibition is relieved and transcription is initiated. That such a signal exists is supported by the observation that a second oral apparatus grafted onto (Tartar 1958), or inserted into (Hyvert 1972), a *Stentor* cell inhibits regeneration following surgical removal of the original OA. The simple picture of a diffusible inhibitor may not be completely accurate, however. Microsurgical studies by de Terra found that cutting the oral apparatus into two pieces but leaving them both attached to the cell (de Terra 1985a), or inverting a section of microtubule rows below an intact oral apparatus (de Terra 1985b) were both sufficient to trigger regeneration. These results suggest that an inhibitory signal may involve the cortical microtubules, either as tracks for motor proteins to carry a signal, or as mechanical elements.

IV. Physics of shape & pattern restoration

Perhaps the most remarkable aspect of regeneration is the ability to restore the complex shapes and patterns of a missing structure. At the tissue scale, this entails forming the tissue or organ into the correct overall shape, but also recovering the distribution of cell types, with the correct arrangement relative to each other. At the cellular scale, shape and pattern restoration entails recovering proper cell polarity, and also recovering the distribution of organelles and other sub-cellular structures, in the correct positions.

Pattern restoration at tissue scale

A major issue in regenerating pattern at the tissue scale is ensuring that the correct cell types are present in the correct numbers, so as to restore proper tissue function. Much of cell differentiation is controlled by molecular signaling pathways, but there is also growing evidence that physical forces can influence cell differentiation to produce different cell types under different physical conditions (Engler 2006; Li 2018). It is not enough to just make the correct cell types, they must be arranged in the correct 3D pattern. In normal development, mechanical forces play key roles in regulating the processes that drive tissue morphogenesis, including oriented cell divisions, rearrangement of cell-cell junctions, and cell shape transitions driving morphogenesis (Tang 2018; Stooke-Vaughan 2018)

Consistent with the idea that tissue regeneration frequently involves the same mechanisms as normal development, these same types of mechanical effects on differentiation and morphogenesis have been seen during regeneration, both in the context of regeneration in an animal and in tissue engineering (Liu 2016; Liu 2022). As with wound healing, electrical gradients can also play a role in directing regeneration (Emmons-Bell 2015).

Pattern restoration at cellular scale

By analogy with the restoration of shape and pattern in tissues, cells also need to restore shape and pattern at two levels: re-creating proper shape and morphology, and placing new organelles and subcellular structures in the correct relative positions.

Perhaps the most fundamental feature of cell patterning is cell polarity, often in the form of an enriched area of actin assembly such as the leading edge of a motile cell. When cells are cut or fragmented, or induced to depolarize, they are able to restore a single site of polarization. Although a number of biochemical models have been proposed to account for the self-assembly of a unique polarization site, based for example on competition for actin or long-range diffusible signals (Altschuler 2008; Wu 2013), mechanical forces also appear to play a central role. When a leading edge assembles, it creates an increase of membrane tension, and this tension has been shown to suppress formation of additional polarization sites (Houk 2012).

Symmetry restoration during regeneration of cells and tissues

In normal development, symmetry breaking events are required to create the body axes in a presumably isotropic egg cell. Removal of part of an animal creates an asymmetrical body, and restoration of normal symmetry is a necessary part of pattern restoration. The restoration of symmetry has been investigated at the tissue scale in juvenile jellyfish (Abrams 2015; **Figure 4A**). When such animals are cut into two pieces, arms do not grow back. Rather, the orientation of the arms shifts, so as to restore radial symmetry (**Figure 4A**). This restoration of symmetry does not involve spatial patterning of cell proliferation or cell death, but instead appears to involve mechanical forces exerted by muscle contraction.

At the single-cell level, symmetry restoration is dramatically seen when the surface is disarranged. The surface of the Stentor cell is covered with parallel microtubule rows, all oriented with the plus ends at the anterior of the cell, near the OA, and the minus ends at the posterior end of the cell, near the holdfast. If the surface of a Stentor cell is randomly slashed with glass needles, the cortex breaks up into randomly oriented pieces of cortex, with microtubule rows running parallel to each other within each such "domain", but with neighboring domains having a random orientation (**Figure 4B**). Over time, normal patterning is restored with the cell covered with parallel rows all running with the same orientation (Tartar 1961). Two proposed mechanisms for restoration are rotational movement of domains until they lock into association with adjacent domains having the same orientation, and biased growth of some domains combined with shrinkage of others, until one orientation wins out and covers the whole cell. The latter mechanism would be reminiscent of domain growth in ferromagnetic materials.

Topological defects in regeneration of cells and tissues

Positional cues generally rely on prior polarization of a tissue or cell such that one region becomes a source of information to direct the development of other regions. When the normal source of such a polarized positional cue is removed, it must somehow be regenerated. In the case of hydra, positional information is encoded in the pattern of transcellular actomyosin fibers that run the length of the whole organism (**Figure 5A**). These fibers appear to dictate the anterior-posterior polarity of the organism during regeneration from explants of the body (Livshits 2017), such that the head and tail form at the edges of the explant where the actomyosin fibers end. It does not seem to be simply that the ends of the fibers dictate position, rather, it is more likely to be the topological discontinuity that results when all the ends come together. This conclusion was reached by experiments in which an explant folds such that some of the fibers abut another set of fibers running in a different direction. These regions of topological discontinuity serve to induce the formation of second heads (**Figure 5A**). In a sense, topological breaks in a patterned surface act as "mechanical morphogens" (Braun 2018).

The ability of topological defects to act as positional cues has also been demonstrated at the single-cell level in Stentor. When topological defects are introduced into the cortex, either by surgery (Tartar 1961) or by RNAi of tubulin (Slabodnick 2014), ectopic holdfasts are observed to form on the cell surface (**Figure 5B**). One model to explain this phenomenon is that a molecule that specifies holdfast formation may traffic on microtubules using a minus end directed motor. In normal cells, all the minus ends are clustered together at the posterior, so the holdfast forms at that spot. When a defect is introduced, the holdfast determinant is transported to the site of the defect leading a new holdfast to sprout.

The idea of a topological discontinuity serving as a positional cue is appealing because it does not require a specific chemical signal to be created. Instead, it relies on the well-known fact that a nonvanishing vector field cannot be mapped continuously onto a sphere. In the biological contexts mentioned, the surface can be viewed as covered with oriented structures determined by a vector field giving the orientation of the structures at each point, and it is therefore the case that at some point on the surface the vectors must vanish (**Figure 5C**).

V. Conclusion

Given that regeneration, as with all developmental processes, represents an interplay of molecular regulatory programs and physical forces, can we say anything about when, or in what contexts, the two types of processes are particularly useful? Certainly, pattern formation via differentiation of one region relative to another seems most easily accomplished by means of molecular processes, involving cell-cell communication or long-range morphogens. On the other hand, physical forces may be particularly adaptable to generating or recovering symmetry in a structure, by means of force balance.

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Figure Legends

Figure 1. **Examples of regeneration across biological scales.** Top left: regeneration of appendages in the Axolotl. Top right: regeneration of appendages (flagella) in the unicellular green alga Chlamydomonas reinhardtii. Bottom left: whole-body regeneration after bisection of Hydra. Bottom right: whole-body regeneration after bisection in Stentor, a single-celled ciliate.

Figure 2. **Wound closure across scales.** A. Two modes of wound-closure at the tissue scale: contraction of a trans-cellular actomyosin "purse string" and filling of the gap by cell migration. B. two modes of wound-closure at the cellular scale in Xenopus oocytes: contraction of the wound margin by an actomyosin purse string, and filling the gap by migration and fusion of membrane vesicles to the cell surface. C. Mechanical modes of wound healing in Stentor: contraction, cytoplasmic retrieval, and twisting (Zhang 2021).

Figure 3. **Triggering regeneration across scales**. A. crustacean eyestalk regeneration repressed by neuropeptides (known as Molt-Inhibiting Hormone or MIH) produced by the X organ in the eyestalk, which signals to the Y organ to inhibit it from triggering molting and regeneration. Severing the eyestalk removed the inhibition, allowing regeneration to proceed. B. Model for regulation of expression of flagella related genes in Chlamydomonas reinhardtii by a repressor sequestered in growing flagella. Proteins are imported into the flagellum by a kinesin-based transport system known as intraflagellar transport (IFT). C. Details of intraflagellar transport. A kinesin moves a complex of proteins into the flagellum and out to the tip. Most of the transport proteins are returned by a dynein motor, but the kinesin returns by diffusion.

Figure 4. **Restoring pattern and symmetry across scales**. A. symmetry restoration in jellyfish by mechanical re-orientation of arms in bisected pieces (based on Abrams 2015). (B) pattern restoration in Stentor cells in which the cortical microtubule rows are mechanically disrupted (after Tartar 1961). C. two modes for pattern restoration in disarranged Stentors: rotational alignment of domains versus domain growth.

Figure 5. **Topological defects as a positional signal across scales.** A. Topological discontinuity in hydra (based on Livshits 2017). When a segment of a hydra is cut out, it folds over itself. In the course of this folding, the actomyosin fibers that normally run in parallel along the body can meet at incompatible orientations, producing a discontinuity. A second head forms at such sites. B. In Stentor, breaks in the longitudinal microtubule bundles that run along the cell lead to formation of ectopic posterior holdfasts (Tartar 1961; Slabodnick 2014). C. Mapping a vector field onto a sphere always results in discontinuities. Red arrows depict a vector field mapped onto the surface of a sphere with the magnitude of the vectors becoming zero at the top and bottom of the sphere.

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