material? Overexpression of the specific engulfment receptor Tim-4 improves the ability of phagocytes to clear apoptotic cells [16–18] and this also increases the upregulation of Ucp2 [6]. While the pathways emanating from Tim-4 and leading to specific Ucp2 upregulation are at present unknown, this piece of evidence connects a plasma membrane receptor with a specific metabolic event at the mitochondrial level, showing that these organelles are integrated with the cellular signaling cascades.

The work by Ravichandran and colleagues [6] adds a novel function for mitochondria in the sequential activation of the engulfment machinery in phagocytes. We expect that this paper will open new exciting avenues of research that will address the many questions raised by these findings. For example, how is ingestion of apoptotic cells coupled to the reported increase in $\Delta\psi_m$ in phagocytes? How can Ucp2 overexpression augment the engulfment ability of these specialized cells? Is this simply linked to energy dissipation and heat generation, or is it a consequence of the ensuing local depletion of ATP that is consumed by mitochondria in a futile attempt to maintain their membrane potential in the presence of a proton leak? Ravichandran and colleagues [6] for now help us in placing mitochondria not only as key regulators of apoptosis execution, but also as essential modulators of the clearance of apoptotic cells.

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Developmental Biology: Physics Adds a Twist to Gut Looping

Much of the effort in understanding the dynamic process of development has focused on dissecting biochemical pathways. Recent studies illustrate that simple physical forces are also important in patterning organs.

Rima Arnaout[1,2,*] and Didier Y.R. Stainier[1]

> "Cell and tissue, shell and bone, leaf and flower, are so many portions of matter, and it is in obedience to the laws of physics that their particles have been moved, moulded and conformed."
> — D'Arcy Wentworth Thompson, *On Growth and Form* (1917)

Nearly a century ago D'Arcy Wentworth Thompson argued that the morphological variations seen among species obey basic physical and mathematical laws (Figure 1). The fantastic spectrum of form and function has inspired the study of allometry — differential growth of tissues from a basic body plan — in many contexts, from tree height to the design of carnivores' footpads [1,2]. As different as these examples are, all of these organisms start as a single fertilized cell. Development is the process that exhibits the dynamic movements and morphological changes that underlie Thompson's observations. Over more than one hundred years, biologists have made considerable progress in understanding development. They have discovered many biochemical regulatory networks that are highly conserved among species and that, taken together, are beginning to provide a coherent genetic blueprint for development [3,4]. They haven't forgotten, however, that development — and life — takes place in a physical world and, as Thompson wrote, obeys physical laws. Examples can be seen in heart tube looping, brain folding, airway branching and gut looping [5–9].

In recent years, researchers have been working to incorporate the effects of physical forces into existing genetic and biochemical models of cell behavior. This work has focused mainly on mechanotransduction: pressure-sensitive membrane proteins, cytoskeletal elements and extracellular matrix components that facilitate the interchange between mechanical forces and biochemical signals on a cellular scale [10]. But what about the scale of a whole tissue? Do the forces created from mechanotransduction across a large group of cells assume a 'life of their own', such that tissue-scale forces can play a separate and definable role in development? A recent paper [11] on gut looping provides strong evidence that the answer is 'yes'.

Tissue-Scale Forces Help Pattern the Gut

In vertebrates, the embryonic gut tube forms the intestines by way of characteristic looping after an initial 270° rotation. Number and size of loops are highly consistent in a given organism [11]. In contrast to the initial rotation [12–14], the factors that drive subsequent gut looping have been unclear. In their recent paper, Savin *et al.* [11] present several hypotheses to account for gut looping in the chick embryo. In their first set of experiments, they found that the number and structure of loops remained similar even as the embryo grew, making it unlikely that body cavity constraints affected looping. They also found that the number of mitotic cells in the gut tube was uniform, making it less likely that differential growth at certain places in the gut tube created the loops.

These findings led the researchers to examine the behavior of the gut tissue on a larger scale by performing several different dissections of the gut tube and the dorsal mesentery, the tissue connecting the gut to the rest of the embryo. What they discovered was that when the gut tube and mesentery were dissected as a unit, the gut retained its looped structure. When they were separated, the gut instead uncoiled into a long, straight tube with a circular cross-sectional shape, while the mesentery relaxed into a thin, uniform sheet. Furthermore, if the gut tube was divided from the mesentery *in ovo* before the initiation of the looping process, loops never formed. They therefore hypothesized that the tethering of the gut to the mesentery was required for looping. Since the dissected gut and mesentery were found to have such apparently simple structures — a tube and a sheet — Savin *et al.* [11] modeled the interaction of the two structures mathematically.

To test the validity of their equations, the researchers set out to create a

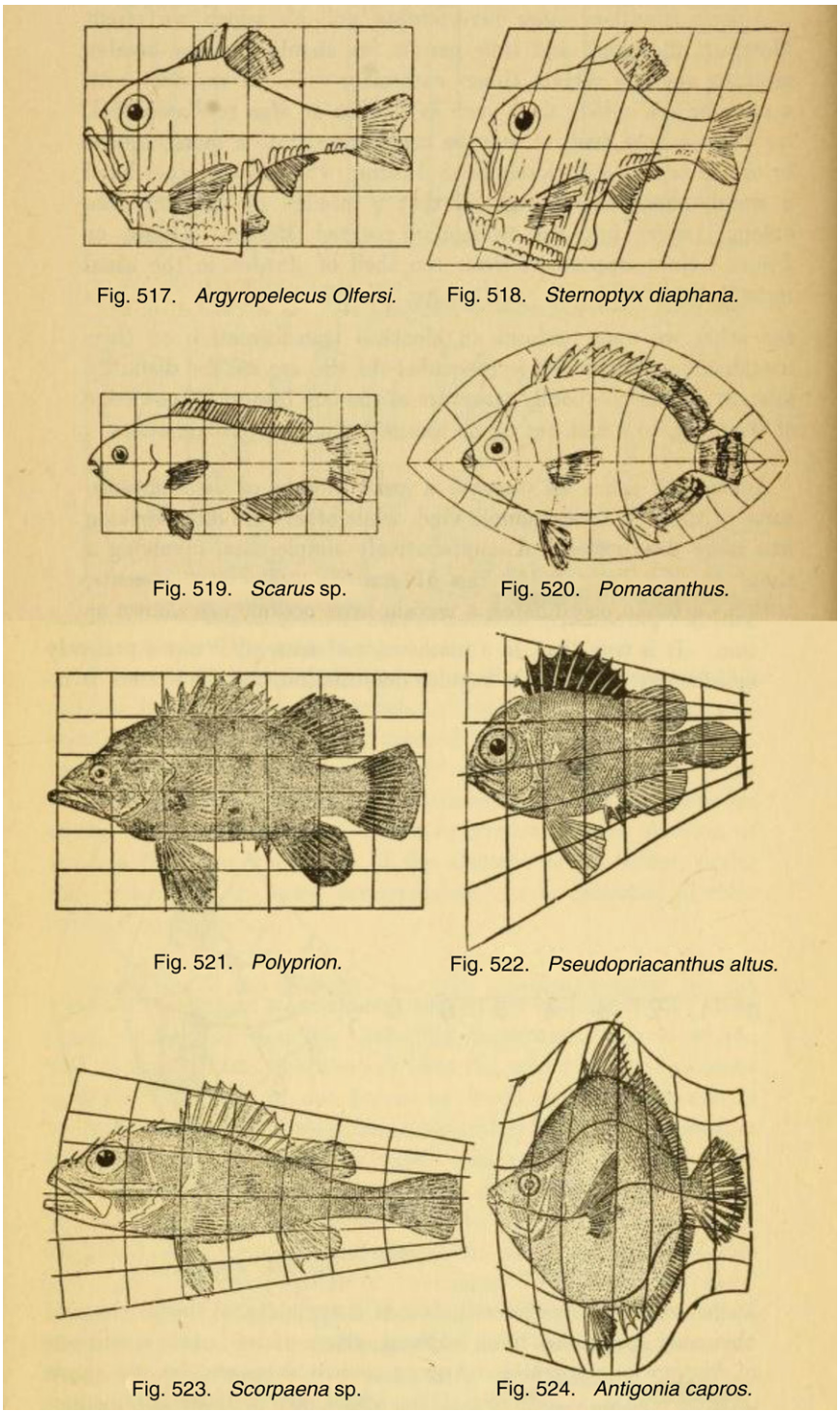

Figure 1. Transforming growth.

In his seminal book *On Growth and Form*, D'Arcy Wentworth Thompson theorized that differently shaped structures in nature were the result of differential growth of tissues based on a largely conserved body plan. Reproduced with permission from [20]. Thompson, D'Arcy Wentworth. *On Growth and Form*, Cambridge University Press, (1917) pp 1062–1063.

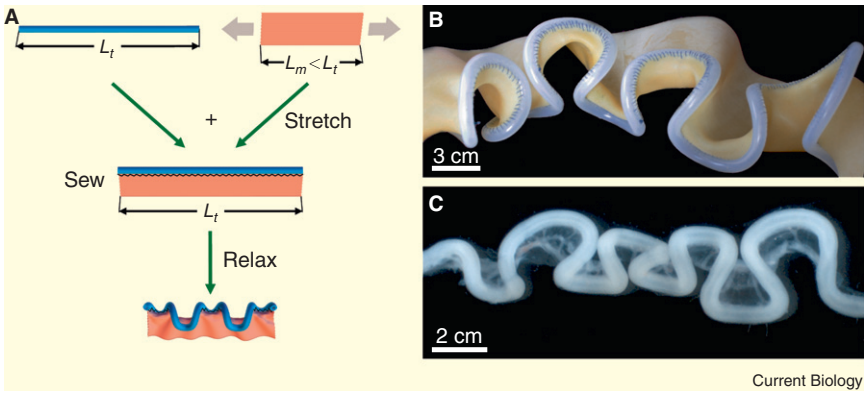

Figure 2. Looping the gut.

A mathematically designed model of the chick gut tube, in blue, and mesenteric sheet, in pink (A) forms a looped structure when fabricated from elastic materials (B) that closely resembles the embryonic chick gut (C). L_t: length of gut tube; L_m: length of mesentery. Modified with permission from [11].

physical model of the gut and mesentery as a rubber tube and a synthetic elastic sheet. To select materials with the right properties, they obtained measurements for the gut tube radius, mesentery thickness, and other parameters from histological sections. They measured the elasticity and tensile strength of the embryonic gut by determining the amount of magnetic force it took to make a small steel ball deform the tissue.

Armed with the tissue-specific values for their equations, Savin *et al.* [11] fabricated a model of the embryonic chick gut tube and mesentery that recapitulates, on the basis of physical parameters alone, the same number and amplitude of gut loops observed in nature (Figure 2). They then made measurements on embryonic quail, finch, and mouse guts, and found that when they substituted these species-specific parameters into their model, it produced the number and amplitude of loops characteristic of those animals. Taken together, these data show that tissue-scale forces have a definite and predictable role in patterning gut looping, seen across different species.

What More Is Needed to Investigate the Physics of Development?

While there has been much study of biomechanical signaling on a cellular scale, the paper by Savin *et al.* [11] is one of few to analyze the effects of forces at a tissue scale and relate those forces to developmental patterning. The idea that simple physical forces, not just gene expression, can affect development is an important advance in developmental biology. It is unlikely that the gut will prove unique in its reliance on tissue-level forces to aid in development [10,15].

Moving forward, it will be important to consider when and where during development these forces might be expected to play a role. In the gut, tissue-scale forces become important only after gut and mesenteric tissues have grown different enough in size to exert significant tension on each other. Later on, however, fibrous attachments limit their ability to move freely and act like the simple tube and sheet modeled in this paper. If patterns can be seen as "diagrams of underlying forces", as D'Arcy Wentworth Thompson wrote, then can we image and diagram the movement of embryonic tissues on the computer to help screen for developmental events that fit simple physical equations [16,17]?

It is also increasingly important to define the tissue parameters that transform mathematical theory into models that can be tested experimentally in biological systems. A strength of the paper by Savin *et al.* [11] is that quantitative measurements of dimension, stress, and strain were used in the equations to show that the rubber model could in fact match the *in vivo* developmental process of gut looping across several species. Existing techniques for measuring material properties of biological tissues [10,15,18,19] will hopefully allow for construction of a database for material properties of different tissues *in vivo*, similar to the extensive gene sequence and expression databases that have facilitated developmental biology research so far.

Finally, while uncovering a role for larger-scale forces in embryogenesis is a leap in our understanding of development, more work must now be done to fill in the gap between gene expression and tissue-level developmental events [17,18]. For example, are there instances where a mutation in a cytoskeletal or cell adhesion gene affects gut looping not by noticeable effects on the cell itself, but by changing the stiffness of the mesentery? Generating a seamless understanding of both biochemical and physical cues in development will require an interdisciplinary effort involving biologists, mathematicians, engineers, and others; and this exciting endeavor may well change the shape of the field itself.

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Mitotic Exit Control: A Space and Time Odyssey

The mitotic exit network (MEN), a protein kinase cascade under the switch-like control of the small GTPase Tem1, triggers exit from mitosis in budding yeast. Now it emerges that signals from both Tem1 and the yeast Polo kinase Cdc5 converge onto the MEN kinase Cdc15 to accurately restrict MEN activation to late mitosis.

Marisa Segal

The safe partitioning of the duplicated genome in dividing cells requires that completion of chromosomal segregation precedes exit from mitosis. This mandatory order of events entails the integration of temporal and spatial cues linking mitotic spindle function, cell spatial coordinates and cell cycle control. The budding yeast *Saccharomyces cerevisiae* offers unique insights into this problem. Now a new study by Rock and Amon [1] proposes a mechanistic basis for such integration within a well characterized signaling pathway controlling mitotic exit in yeast.

Budding yeast divides asymmetrically into a mother cell and a bud, with chromosomal segregation occurring across a narrow constriction between the two — the bud neck. In anaphase, spindle elongation begins in the mother cell and proceeds such that one spindle pole and a set of the duplicated chromosomes are delivered to the bud. Only then, spindle disassembly and cytokinesis can follow.

Mitotic exit in yeast is conditioned to the sustained activation of Cdc14, a phosphatase that targets cyclin-dependent kinase (CDK) substrates for reversal of their phosphorylated state [2]. This prompts events leading to inactivation of CDK, the ultimate trigger for mitotic exit [3]. Cdc14 remains inactive when sequestered in the nucleolus. After anaphase onset, two signaling pathways sequentially control Cdc14 release. The FEAR network induces limited release followed by the mitotic exit network (MEN) that signals the persistent dispersal of Cdc14 throughout the cell, bringing about mitotic exit [4].

Activation of the MEN cascade is controlled by Tem1, a small GTPase localized to the spindle pole body (SPB), the yeast equivalent of a centrosome. Tem1 is downregulated by Bub2–Bfa1 (Figure 1), a two-component GTPase-activating protein (GAP) that stimulates hydrolysis of Tem1-bound GTP. This GAP is controlled by two kinases acting antagonistically. Phosphorylation of Bfa1 by the polo kinase Cdc5 inhibits the GAP, rendering Tem1 active, presumably due to high intrinsic GDP–GTP exchange. A second kinase confined to the mother cell, Kin4, antagonizes Cdc5 action, thus indirectly inhibiting Tem1. By contrast, a Tem1 positive regulator, Lte1, is restricted to the bud. Furthermore, correct spindle alignment instructs Bub2–Bfa1 asymmetric build-up at the SPB destined for the bud. In this way, the SPB can sense negative and positive signals compartmentalized in the mother cell or the bud, respectively, as it transits across the bud neck. This surveillance system is known as the spindle position checkpoint [5]. Once the SPB enters the bud, Tem1 escapes Kin4 inhibition and is activated by Lte1, although the precise mode of activation is unclear [6–9]. Active GTP-bound Tem1 recruits MEN components to the SPB, starting with the kinase at the top of the cascade, Cdc15. This is followed by activation of the kinase Dbf2–Mob1, partly responsible for the release of Cdc14 to the cytoplasm, the hallmark of MEN activation [10,11].

Failure to position one pole of the elongating spindle across the bud neck prevents MEN activation and mitotic exit. Yet, in addition to this spatial control, is there a separate input to enforce temporality when spindle position is not disrupted? In other words, is the activation of the MEN inherently restricted to late anaphase in an unperturbed cell cycle and, if so, how is this temporal window set?

In their study, Rock and Amon [1] dissected cell-cycle dependent activation of the MEN in a setup designed to uncover Tem1-independent controls. Accordingly, Dbf2–Mob1 activity (a downstream readout for MEN activation) was still restricted to late mitosis in an *lte1Δ kin4Δ* mutant strain that no longer possesses the spatial cues to regulate Tem1 based on SPB position. Even a strain in which the effector MEN kinase Cdc15 was overexpressed to bypass the complete absence of Tem1 *(tem1Δ CDC15-UP*) retained cell cycle regulation of Dbf2–Mob1, although it lost checkpoint proficiency. Thus, only the checkpoint-enforced delay subject to spatial cues operates via Tem1.