Show of Force Scientists are learning myriad ways that small forces add up to a big impact on the development of organisms, from plants to animals.

BY RACHEL EHRENBERG

ILLUSTRATION BY MARTIN NICOLAUSSON

JENNIFER ZALLEN HAS scrutinized millions of cells, but the day she witnessed a fruit fly cell in a tug-of-war stands out.

Zallen, an HHMI early career scientist at Memorial Sloan Kettering Cancer Center, was exploring the role of mechanical forces in the dramatic elongation that a fruit fly embryo undergoes during its development. Over a mere two hours, roughly a thousand cells mobilize en masse and rearrange themselves into a nascent fly that's half the embryo's original width and twice its length. Previous work had implicated the motor protein myosin in this mass movement, so Zallen's team labeled myosin with a fluorescent tag and rigged a video camera to the microscope so they could watch the protein in action. Then, her postdoc Rodrigo Fernandez-Gonzalez delicately poked the tip of a glass needle into the embryo and sucked in the tiniest bit of fluid, yanking on a nearby cell.

Myosin flooded to the site, enabling the pinned cell to contract and then escape, essentially pulling itself away from the needle. "It was fast," says Zallen, "too fast to involve changes in gene expression in the nucleus." There was no change in the cell's genetic makeup and no chemical signal recruiting myosin to the needle's tip. Yet the cell and its neighbors had exhibited an immediate and collective response to the tug of the suction. Just experiencing mechanical tension appeared to be enough to kick myosin into gear.

Jennifer Zallen thinks that cells might use force as a compass, to help them move in the right direction.

Kevin Wolf

Those experiments, published in *Developmental Cell* in 2009, are part of a growing effort by scientists to elucidate the role of mechanical forces in shaping biological tissues and, ultimately, entire organisms. The research is not only yielding new insights into the stunning aesthetics of animal and plant morphology, but it may also lead to new tricks for controlling plant architecture or for halting cancer's spread.

Scientists have long appreciated the idea that mechanical forces are integral to creating shape and form. Nearly a century ago, in the introduction to his treatise *On Growth and Form*, Scottish scientist D'Arcy Thompson wrote, "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, molded and conformed."

The importance of those physical laws to the formation of healthy tissues and organs has also long been acknowledged: weight-bearing exercise is crucial for maintaining strong bones, and turgor pressure on cell walls of plants keeps leaves and flowers from wilting. But during the molecular revolution of the 1990s, the role of genes, signaling molecules, and proteins in development took center stage, partially obscuring the important role of physical forces.

A revival is now underway. Armed with knowledge gleaned during the molecular era, along with new tools for manipulating and imaging cells and unprecedented computing power, scientists are reexamining the profound impact of force. By zeroing in on cells as they are squeezed and stretched in real time, researchers can untangle the developmental consequences of force generation, propagation, and detection. Efforts to quantify these actions, as well as the cellular players involved, are leading to testable models that may eventually reveal how tissues and fully fledged organisms take shape.

Push and Pull

Some striking examples of the importance of mechanical forces are coming from studies of the developing embryo of that workhorse of the lab, the fruit fly *Drosophila melanogaster*. Unlike plant cells, which for the most part don't move (though there are exceptions), the cells of developing flies and other animals often travel as they realize their ultimate fate.

"During development, cells often move a great distance from where they are born to where they need to end up," says Zallen. "These cells have to navigate through complex mechanical environments, and they are constantly pushing and pulling on each other as they move."

The motor protein myosin II, best known for its role in muscle contraction, has emerged as the primary mediator of this pushing and pulling. Some of myosin II's jobs include helping cells divide and travel as they contribute to the development of tissue-level structures such as grooves and tubes.

When Zallen was a postdoc in the Princeton University lab of HHMI Investigator and Nobel Laureate Eric Wieschaus in the early 2000s, she was investigating how turning on certain genes in a particular spatial pattern could orient cells as the *Drosophila* embryo elongated. Here, myosin II came into play; during elongation, the force-generating protein accumulated at cell borders along the fly's head-to-tail axis. Continuing this work in her own lab, Zallen found that the accumulation and contraction of myosin was driving a

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coordinated, multicellular movement that led to a dramatic change in the embryo's shape.

Zallen began to wonder if cells might detect and make use of the forces generated by their neighbors, inspiring the tug-of-war experiments and cementing the role of mechanical tension in regulating myosin's activity. Her team showed that once a single cell starts to constrict, that force spreads: pulling on neighboring cells causes them to constrict as well, producing a contractile cable that extends across cellular neighbors like a long rubber band.

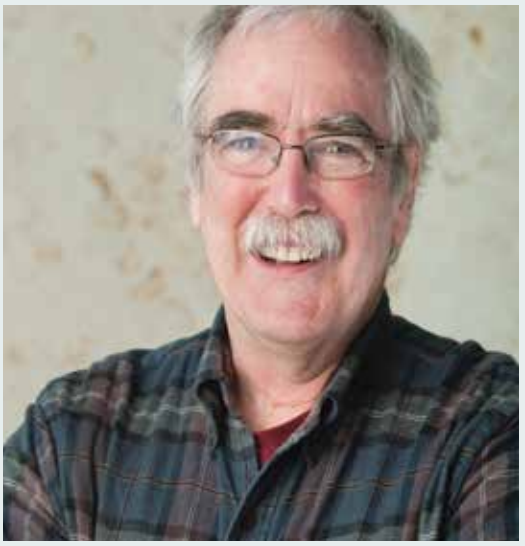
"We're very interested in the possibility that cells could use these forces as a compass to help them move in the right direction," says Zallen. "There is an increasing appreciation that forces can act as signals that influence the shape, fate, and behavior of cells to enable them to assemble into tissues during development."

Zallen's lab group has since shown that as the *Drosophila* embryo elongates, the contraction of these long cables of myosin – and myosin's partner in crime, the cytoskeletal protein actin – draws cells into unexpected intermediate structures, little flower-like conglomerates called rosettes. These rosettes form when cells align into columns and shrink their connected edges together, thanks to the contracting myosin cable that pulls several cells into contact at a single point. The rosettes then disassemble in a direction perpendicular to the way they formed. The process transforms a cluster of cells that started out as tall and thin into one that is now short and wide, promoting elongation of the fly embryo.

Now the researchers are starting to pinpoint various cellular players that guide and respond to the jostling involved in embryo elongation. In 2012, Zallen and her team reported that rosettes don't form properly in embryos that aren't able to make the signaling molecule Abl, an enzyme that enables cells to adhere to each other under tension and execute group cell movements. Her team also discovered the cell-surface proteins that are laid down in bold stripes along the embryo's head-to-tail axis and that guide the direction of cell movements to help make the embryo longer and thinner. This patterning directs myosin's contractile machinery, orchestrating the mass movement of elongation, reported Zallen and colleagues in *Nature* in November 2014.

Wieschaus compares the patterning that precedes force generation in *Drosophila* to imaginary dotted lines on a sheet of origami paper showing where creases need to go to fold it into a bird. "Once you have a pattern, it gives you a way to localize forces; then you can get form," he says. The idea that tissue remodeling might result from the concerted action of assemblies of cells rather than from individual cells is a view of development that's gaining traction, he adds.

"We began by thinking of the problem as a whole bunch of bricks. If you could understand how each brick behaved, you could put that all together and say how it leads to a whole organism," Wieschaus explains. "But over the past couple of years, we've become aware that maybe it's easier – or more useful or more correct – to realize that changes in morphology are bigger than single cells."



Eric Wieschaus's lab team studies how force influences the flow of groups of cells.

Mass Movement

There's growing evidence that networks of contractile myosin cables are a force to be reckoned with during embryonic elongation in organisms other than the fly. In 2012, for example, Gerd Walz of the University Hospital Freiburg in Germany and HHMI Early Career Scientist John Wallingford of the University of Texas at Austin and colleagues showed that myosin cables play a crucial role in the elongation of kidney tubules in two model vertebrates, the frog *Xenopus* and mice. Other research, by Masatoshi Takeichi and colleagues at the RIKEN Center for Developmental Biology in Japan and Ann Sutherland and colleagues at the University of Virginia, revealed a similar role for myosin cables during the development of the chick and mouse neural tube.

Recent work also implicates physical forces at work in metastatic cancer. Research from Valerie Weaver's lab at the University of California, San Francisco, as well as in

Kevin Wolf

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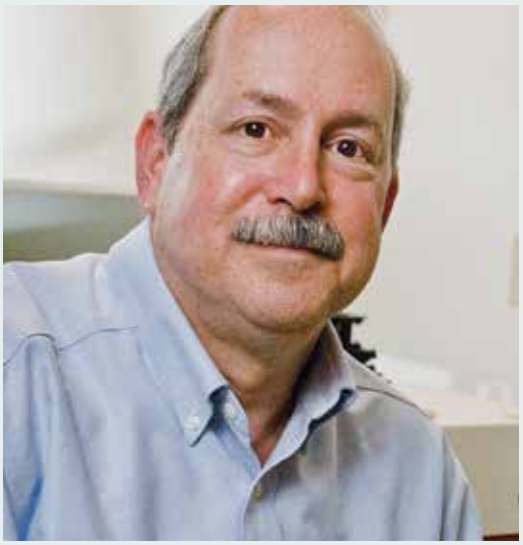
labs elsewhere, has found that the physical stiffness of the extracellular matrix, a web of fibers outside the cells, plays a prominent role in the aggressiveness of breast cancer. Enhancing the mechanical stiffness of this matrix activates a protein that aids the tumor's ability to spread, Weaver and colleagues reported in *Cancer Research* last year. The stiffness, which puts physical tension on epithelial cells, also drives malignancy by downregulating the cells' production of an important tumor suppressor protein, Weaver and her colleagues recently reported in *Nature Medicine*.

A view of cells as social constructs, which respond to mechanical cues en masse, might lead to ways to interfere with those cues when they're implicated in disease, says Zallen. "It may be useful to think about the metastasis of certain cancers as a group activity," she says.



Elliot Meyerowitz investigates the impact of mechanical stress on growth in plants.

Wieschaus has taken this holistic approach to the extreme. His lab had been investigating the development of the ventral furrow, an inward folding of cells that characterizes the transition of the *Drosophila* embryo from a single sheet of cells into three germ layers that ultimately differentiate into adult organs and tissues. This stage occurs right after the cell membranes form, when the fly-to-be transforms from one giant cell with many nuclei to an embryo of 6,000 cells.

Working with his Princeton colleagues Oleg Polyakov, Konstantin Doubrovinski, and Bing He, Wieschaus developed an approach that uses two-photon imaging and fluorescent beads to track the flow of cells as the ventral furrow forms. The researchers discovered that the infolding results from mechanical forces in the form of pulsing contractions driven by myosin on the cell surface. Mathematical modeling revealed that the cells were flowing much like a viscous fluid – behavior captured in the tidy Stokes equations of physics, which describe the flow of such fluids as paint and lava. This raised the question of how ventral furrow development would proceed if the embryo weren't partitioned into the individual units we call cells.

To investigate the forces in the absence of cells, Wieschaus's team knocked out two genes – *slam* and *dnk* – that direct the development of the cell membranes. Remarkably, they found that, during ventral furrow formation, a fruit fly embryo without cell membranes behaved very similarly to one with cell membranes. As the team reported in *Nature* in April 2014, the process proceeded in a messier and slower fashion than it did in an embryo with cell membranes, but the flow patterns were essentially the same, upending the cell-focused view.

"As cell biologists, we believe that cell membranes are really important for everything," Wieschaus says. "But we found that we could eliminate all the partitioning, have this big goo of cytoplasm flowing like a fluid, and yet the embryo goes on and does its stuff."

A simple model of mechanical forces via myosin constriction could account for the changes in shape that lead to ventral furrow formation, Wieschaus says. Similar squeezing at a cell's apical end occurs during folding in other places and other embryos, including during the development of the *Drosophila* respiratory system and the closure of the *Xenopus* neural tube. Such commonalities suggest that transmitting force via viscous flow might be another fundamental mechanism for establishing form.

Pattern and Form

Mechanical forces may also explain patterns observable with the naked eye, including an enduring mystery of plant architecture. While many animals undergo a massive rearrangement of cells to form an embryo that then, in essence, simply enlarges, plants' growth is often indeterminate – that is, they can add new leaves, branches, and flowers until death. This new growth isn't haphazard; it follows a conspicuously regular pattern that's observable by looking, for example, head-on at the tip of a new shoot. It turns out that mechanical forces generated in the hotbed of plant embryonic growth – the shoot apical meristem, a concentrated region of dividing cells – are crucial in driving this predictable geometric morphology.

Ever since the ancient Greek scholar Theophrastus noted this striking regularity in the arrangement of leaves around a plant stem, botanists, physicists, and mathematicians have been trying to explain how such systematic placement, called phyllotaxis, arises. (More modern-day scholars who have studied the phenomenon include D'Arcy Thompson, who noted the crisscrossing patterns that form spirals in pinecones and sunflower heads.) It was phyllotaxis that turned the attention of Elliot Meyerowitz, an HHMI investigator at the California Institute of Technology (Caltech), toward the role of mechanical forces in generating form.

Using live imaging techniques, Meyerowitz and his colleagues had been investigating how differing concentrations of the plant hormone auxin related to patterns of plant growth. It had long been known that auxin was crucial in determining where each new flower or leaf appeared on a plant; experiments dating back to the 1930s demonstrated that daubing a paste of auxin onto the meristem prompted the growth of new plant organs. So Meyerowitz – with a team that included his then Caltech colleagues Bruce Shapiro and Marcus Heisler, plus Henrik Jönsson, then of Lund University in Sweden, and Eric Mjolsness of the University of California, Irvine – began tracking the concentration of auxin in meristem cells of the model plant *Arabidopsis*.

Previous work in several laboratories had suggested that the membrane protein known as PIN1 was probably an auxin pump, controlling the direction of the hormone's flow out of

Eric Reed

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cells in the plant shoot's meristem. But PIN1's location isn't fixed in cell membranes: the pumping protein can move around, and something was directing it to membrane regions that were adjacent to cells already high in auxin. Meyerowitz and his colleagues suspected that auxin's influence on the location of the PIN1 pump could lead to a particular spatial pattern of high auxin concentration, and that this might account for the regularity of phyllotactic spirals and whorls. If cells somehow sensed their own high auxin and recruited PIN1 to the nearest membrane regions in adjacent cells, the auxin concentration would increase even more in the original cell. This would induce growth of a new leaf or flower. But as auxin concentrations increased locally, neighboring cells would become depleted in auxin, leaving a spot with no leaf or flower. Cells farther away from this auxin-depleted site would, by comparison, have more auxin and thus would recruit PIN1 to attract more auxin and again induce a new leaf or flower.

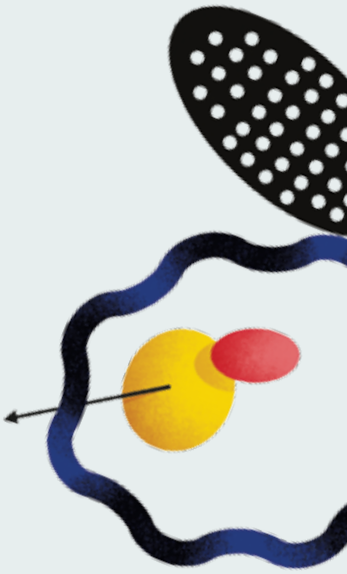
The researchers created a mathematical model that incorporated this proposed feedback mechanism. When they ran computer simulations of the model, auxin peaks emerged at regular distances, capturing the regular patterning of phyllotaxis – a finding published in *Proceedings of the National Academy of Sciences* in 2006.

But mysteries remained, including how the plant cells were sensing local auxin concentrations. The hormone was known to weaken plant cell walls, leading the team to wonder whether mechanical stress might signal to cells that they were adjacent to neighboring cells high in auxin. This, in turn, would recruit PIN1 to the region of the cell membrane nearest the high-auxin neighbor.

In a series of elegant experiments, including ones in which the scientists weakened or obliterated particular plant cell walls with a laser, mechanical stress indeed emerged as the mediator of PIN1's dynamic behavior. They found that as PIN1 directed the flow of auxin from an area of low to high auxin concentration, the cells highest in auxin expanded. In plants, cell walls are shared. So it appeared that mechanical stress on a common wall alerted the cellular neighbor that auxin concentration was high nearby, thus bringing in PIN1.

"Basically, all the observations were in the literature, but no one had thought to put stress into the equation," says Meyerowitz, who published the findings with Marcus Heisler and other colleagues in *PLOS Biology* in 2010.

More recently, Meyerowitz and his collaborators have shown that mechanical stress plays an important role in shaping plant cells beyond the meristem. It turns out that the puzzle-shaped pieces of the so-called pavement cells on a leaf's surface create intracellular stresses that cause reorganization of the cytoskeletal proteins called microtubules. These proteins then help dial up the production of cellulose, which, in turn, reinforces the cell walls against the stress, the team reported in *eLife* in April 2014. Combining the microtubule findings with the auxin-related research yields a simple model of feedback driven by physical forces: "Mechanical stress tells cells how to grow, and cell growth creates mechanical stress – and morphology," Meyerowitz and his colleagues wrote in a 2014 *Current Biology* review paper.



Other labs are finding evidence of physical stressors as well. For example, Audrey Creff and Gwyneth Ingram of the Laboratoire de Reproduction et Développement des Plantes in Lyon, France, recently showed that a mechanically sensitive layer of cells in the seed coat of *Arabidopsis* responds to stress exerted by the seed's nutritive tissue, the endosperm, resulting in a fine-tuning of seed size.

The Meyerowitz lab is now looking for cellular stress sensors responsible for the cell wall effects on PIN1 and for the different sensors that mediate the effects of stress on the cytoskeleton. He believes that a greater understanding of the relationship between mechanical force and plant growth is important not just for elucidating a plant's current growth patterns; the research may also lead to techniques for engineering superior food crops – for example, produce with modified leaf arrangements that maximize photosynthesis in a particular growing region.

"Now that we're beginning to understand the feedback between physical stress and growth, it may give us a new way to intervene – or at least predict what would happen if we change things," Meyerowitz says. And for developmental biology in general, bringing mechanics back to the fore may help resolve older mysteries of shape and form.

"As people start to look at things in terms of physical signaling, not just chemical signaling, it may explain quite a bit," he observes. "It seems like we can make some rapid progress in solving old problems." ■