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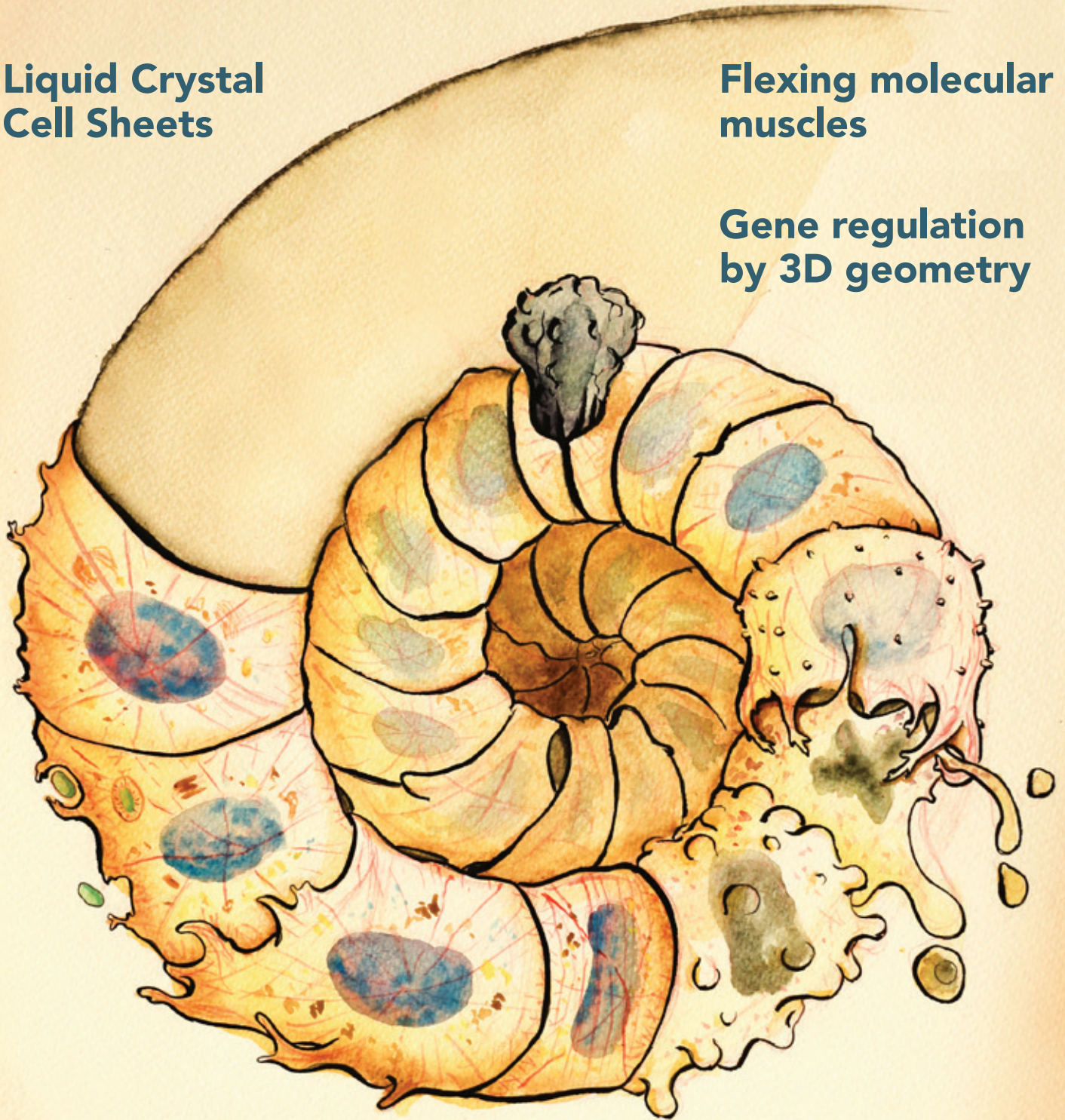
MBINSIGHTS

DECODING THE LIVING MACHINE

**Liquid Crystal
Cell Sheets**

**Flexing molecular
muscles**

**Gene regulation
by 3D geometry**



100 years shaping Mechanobiology
Looking back at 'On Growth and Form'

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At MBI, we are exploring mechanotransduction through four major research programs:

Molecular Mechanobiology

Investigating how groups of proteins come together to form modular functional units that are capable of mediating diverse cellular functions by sensing and relaying mechanical signals between various components of the cell.

Cellular Mechanotransduction

Deciphering how a cell's behavior within a tissue is guided by its communication with neighboring cells and the extracellular matrix through the formation of protein-based adhesion complexes.

Tissue Mechanics

Applying biophysical principles to study the highly-coordinated orchestration of cellular events in a tissue, and understand its relevance during the development of an embryo as well as during tissue repair in adult organisms.

Technology Innovation

Engineering novel platforms for experimentation at various levels, from whole organisms down to individual proteins.

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FROM THE DIRECTOR

2017 has seen significant growth in the field of Mechanobiology, both from within the MBI, and in biophysics labs around the world. Significantly, this year also marks 100 years since the publication of 'On Growth and Form', the magnum opus of the great mathematician and biologist Sir D'Arcy Thompson. Published in 1917, this work articulates the earliest suggestions of a physical basis for biological form and function, and is often recognized as the founding work of the biophysical sciences.

The cover illustration chosen for this volume of MBInsights magazine marks the significant influence Thompson's ideas have had on the establishment of mechanobiology, and indeed biology in general. The accompanying cover story explores how quantitative tools and techniques applied at the MBI continue to test the relevance of Thompson's ideas to modern biophysical principles.

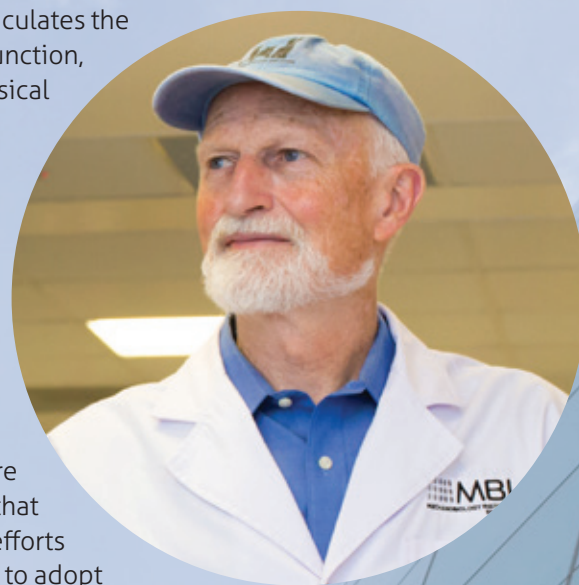
Also in this volume of the institute's research magazine we feature synopses of several exciting discoveries and innovations that stemmed from the collaborative, multidisciplinary research efforts carried out at the MBI. One of our main objectives at the institute is to adopt an integrated approach, to quantitatively describe the molecular mechanisms driving dynamic cellular processes. On one hand, physicists have been instrumental in the development of tools and techniques that are used to manipulate the physical parameters of the cellular microenvironment, and then quantitatively assess the responses at various levels, from the whole cell down to single proteins. The huge data sets that are generated from these experimental studies are analyzed mathematically by theorists, who then build testable models and simulations based on the data. A selection of studies that employed such interdisciplinary approaches were published in high-impact biomedical journals over the past year, and have been summarized in this volume of MBInsights.

Specifically, we have described how advanced super-resolution and light-sheet microscopy methods are employed to characterize the nanoscale architecture of cadherin-based cell adhesions and the dynamics of nuclear proteins, how distinct mechanisms exist to expel dying cells from epithelial cell sheets, and how actin remodeling regulates cell shape changes in normal epithelial tissue and leads to the formation of vesicles that transports excess bile out of the liver during liver diseases such as obstructive cholestasis.

The breadth of the cellular processes studied by MBI's researchers, which range from single molecule dynamics and intercellular communication, to the development of multicellular organisms, is clearly owing to the expertise of its interdisciplinary research community. We hope to see further growth in this community through recruitment of new faculty, postdocs and students. In addition, we invite you to join us in collaborative studies at the MBI. In this way, new questions on biological systems will continue to be answered using approaches and expertise from not only traditional cell biology, but from the theorists, physicists and engineers.

Professor Michael P. Sheetz,

Director
Mechanobiology Institute, National University of Singapore



MBINSIGHTS DECODING THE LIVING MACHINE

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MBINFO DEFINING MECHANOBIOLOGY

For more information about mechanobiology, visit MBInfo (mechanobio.info), the flagship project of the Science Communications Unit. It provides review-style descriptions of the mechanics underlying various cellular processes. In addition to describing the basic concepts in Mechanobiology, the resource also provides up-to-date information by including the latest findings in the field. The descriptions on MBInfo are supplemented with high-quality illustrations and animations that are produced in-house. The wiki-style content of MBInfo allows for easy editing of the website and encourages active contribution from students and scientists around the world.

Visit MBInfo yourself, and contribute to this growing community!

What is Mechanobiology?

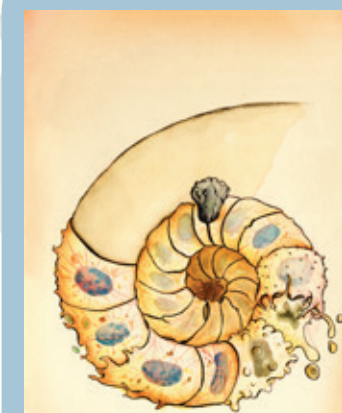
Mechanobiology describes how physical factors, such as forces and mechanics, are able to influence biological systems at the molecular, cellular, and tissue level. The fundamental process which drives mechanobiology is mechanotransduction, the ability of cells to convert mechanical stimuli into biochemical signals.

For example, a cell can sense and respond to the three-dimensional physical properties of its environment. These parameters include matrix density, geometry, and substrate rigidity. After sensing these mechanical stimuli, the cell can convert them into biochemical signals which enables specific cellular responses such as migration, proliferation, and differentiation.

The Mechanobiology Institute

Founded in 2009, the Mechanobiology Institute was created through joint funding by the National Research Foundation and the Ministry of Education with the goal of creating a new research centre in mechanobiology to benefit both the discipline and Singapore.

At the Mechanobiology Institute, National University of Singapore, our goal is to develop a new model of biomedical research by focusing on the quantitative and systematic understanding of dynamic functional processes. With a systems-level perspective we are working to identify, measure and describe how the forces for motility and morphogenesis are expressed at the molecular, cellular and tissue level.



About the Cover:

The cover reimagines the classic image of a Nautilus shell from On Growth and Form. Here, the chambers of the shell have been replaced by cells undergoing processes commonly studied in mechanobiology, e.g. extrusion, phagocytosis, apoptosis, blebbing, and contraction. For more details, please read the feature "100 years of On Growth and Form" on pages 30-33.

Cover illustration by Diego Pitta de Araujo.

A CELLULAR SHOCK ABSORBER

HOW THE STRUCTURE OF TALIN MAKES IT AN IDEAL FORCE BUFFER

Written by Andrew Wong and Lakshmi Ramachandran.
Illustration by Diego Pitta de Araujo.

A research team from the Mechanobiology Institute (MBI), National University of Singapore, and the University of Kent and University of Cambridge in the UK, show how a protein called talin senses mechanical force exerted on a cell and buffers the force through stochastic unfolding and refolding of its multiple folded domains during mechanical stretching and relaxation of talin. They reveal a novel role for talin as a cellular force buffer that defines the physiological range of force acting on all the proteins in the talin-mediated force transmission pathway, which is crucial for the mechanical regulation of various cellular processes. This study is published in Nature Communications.

Talin buffers cellular forces

In the body, cells are continuously impacted by mechanical forces. These forces can arise from the external surroundings or are generated internally by dynamic contractions of the cytoskeleton, a filamentous protein network within the cell. Maintaining and balancing a certain level of force

is essential – too much force can lead to physical disruption of the mechanical linkages in the cell, but insufficient force will lead to inactivation of mechanical signalling pathways necessary for cell movement and development.

By combining single molecule manipulation experiments with structural biology, physics-based modelling, and numerical simulation, a collaborative research team led by MBI Principal Investigators Prof. Jie Yan and Prof. Michael Sheetz identified that the protein talin is able to buffer force, effectively acting as a shock absorber for the cell.

Talin links focal adhesions – protein complexes that connect to the external environment – with the cytoskeleton that provides structural stability and the basis for cell movement. Previous studies have demonstrated that talin is 'mechanosensitive', meaning that it can sense and respond to mechanical force, and convert these forces into biological signals. Structurally, talin has a rod-like shape, subdivided into a linear array of 13 domains, which can unfold to allow talin to increase in length several

fold. In living cells, the level of force in talin was recently measured to be between 5-10 pN, a range which allows various crucial mechanosensitive protein-protein interactions take place; however, the mechanism of how this force level is maintained was unknown.

In order to solve this mystery, the scientists used magnetic tweezers to probe the mechanical response of talin at a single-molecule level. The magnetic tweezers were used to apply mechanical force to a single talin molecule, causing the domains inside talin to unfold. By carefully measuring the rates of unfolding and refolding of the domains under different forces, the researchers were able to simulate the force fluctuation during the process of stretching and relaxation of talin.

They discovered that the average force remains within a narrow range between 5-10 pN, even when talin is fully extended to a length of 500 nm. This result robustly explains the recently reported levels of force measured in talin in living cells. Importantly, this force range defines the force threshold for talin dependent mechanosignalling, a critical pathway that drives a number of biological processes, such as maintaining adhesion stability, growth, and maturation.

This study revealed that talin is a highly effective force buffer which functions as a cellular shock absorber. The physical principle revealed for talin

as a force buffer is universal to any protein that is comprised of a linear array of domains.

Interestingly, a set of similar proteins have evolved that link the cytoskeleton not only to the focal adhesions but also to neighbouring cells and the nucleus. The mechanism revealed in this study therefore suggests that all these proteins can buffer force in a certain range to enable robust mechanosensitive interactions. In a similar way to how the suspension of a car connects the tyres to the chassis and enables a smooth ride even on difficult terrain, these proteins connect adhesions to the cell skeleton and absorb force so that the cell remains mechanically stable and active.

ABOUT THE RESEARCHER: JIE YAN

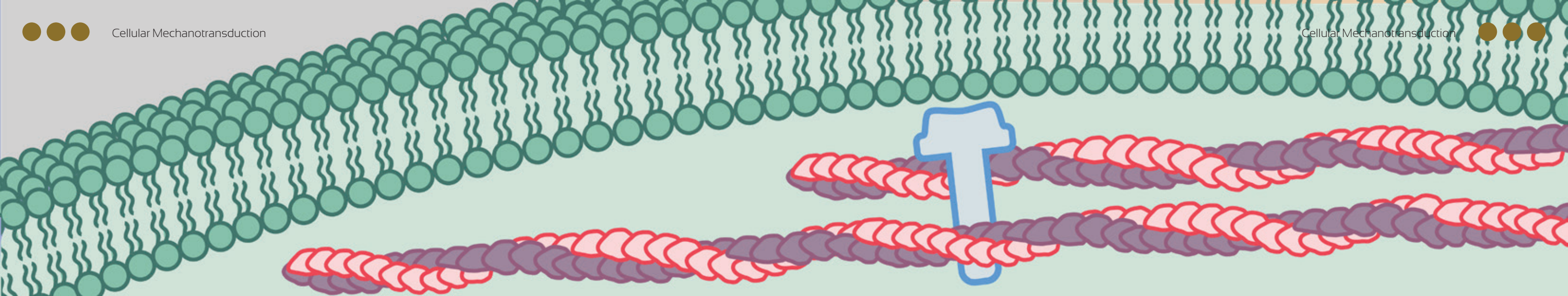


Principal Investigator at the Mechanobiology Institute, Centre of Bioimaging Sciences, and Professor at the Department of Physics, NUS. The Yan lab uses advanced single-molecule manipulation and imaging technologies to investigate molecular mechanisms of mechanosensing, and DNA structure and function.

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REFERENCE:

Yao M, Gault BT et al, The mechanical response of talin, Nat. Comms, 7:11966, doi: 10.1038/ncomms11966.



A MOLECULAR RIVET FOR LONG-RANGE FORCE TRANSMISSION

Written by Steven Wolf with Ding Wei Yung. Illustration by Melanie Lee.

Researchers from MBI, National University of Singapore have described, for the first time, how plastin, an actin-bundling protein, acts as a molecular rivet, providing global connectivity to the cortex underlying the plasma membrane of embryonic cells to facilitate polarization and cell division. This work was published in the Journal of Cell Biology.

All multicellular organisms begin their life when a sperm cell fuses with an oocyte. The newly fertilized zygote must then undergo countless rounds of cell division as it forms an embryo. Some divisions are symmetric and generate two identical daughter cells in a process known as

cytokinesis. Others are asymmetric, and result in unequal daughter cells that differentiate into distinct cell types with specialized functions. Before asymmetric division, these cells undergo a process known as polarization, where the top or front of the cell contains a different set of protein-based structures and machines to the bottom or back of the cell.

Both cytokinesis and polarization are driven by a contractile component of the cell called the 'cortex'. This thin layer is located inside the cell, immediately adjacent to the plasma membrane and is primarily composed of actin filaments (F-actin), which are cable-like structures that are dynamically assembled and disassembled, and maintain cell shape. The cortex also contains the motor protein non-muscle myosin II, which confers contractility to the network, and other actin-binding proteins such as those that bundle filaments together or facilitate the assembly of new filaments. Despite the structural arrangement of the cortex being well established, it was unclear how all of these proteins work together in a living organism to produce higher order actin-based structures, and transmit mechanical force during crucial developmental processes.

To investigate these questions, a team of interdisciplinary scientists from MBI, the Institute of Molecular and Cell Biology, A*STAR, Singapore, and European Molecular Biology Laboratory, Germany,

examined the cortex in a developing organism, specifically, *Caenorhabditis elegans* (*C. elegans*), which is a 1mm long transparent nematode worm. What became apparent from the investigation, which was led by Asst. Prof. Ronen Zaidel-Bar, was the important role of an actin-binding protein called plastin (a.k.a. fimbrin) in early *C. elegans* development.

Plastin binds to actin filaments to facilitate their bundling and strengthen the actin filament network so that it can withstand the forces generated during filament contraction, and also those applied to the cortex from external stimuli. By functioning as a molecular rivet, plastin enabled the cell cortex to function efficiently, thereby facilitating polarization and cytokinesis. Using microscopy, genetic and computer modelling approaches, the researchers examined the cell cortex during the earliest stages of *C. elegans* development, with a particular focus on plastin. Although previous efforts had identified plastin within the cortex, none had revealed its full importance in the development of an embryo.

To begin the investigations, MBI PhD candidate Wei Yung Ding examined zygotes from a mutant strain of *C. elegans* where the function of plastin had been lost. In these zygotes, both polarization and cytokinesis were disrupted to the point that they either did not occur, or were significantly delayed. With the

ability to observe how *C. elegans* formed with plastin mutated, Ding et al turned their attention to examining contractility within the cortex, during *C. elegans* development.

From Isolation to Global connectivity

To do this the researchers examined the organization and dynamics of non-muscle myosin II at the cortex during polarization. In normal zygotes, the myosin motor proteins will accumulate into large clusters that generate large contractile forces. However, when plastin function was lost, the researchers noted that the myosin did not accumulate in clusters as it does in normal cells. This meant that the contractions generated were much weaker compared to normal cells. Ultimately, the loss of strong, coordinated cortical contractions in the plastin mutant worm embryo resulted in defective polarization. This was evident from the disrupted separation of two proteins that would otherwise accumulate at either end of the cells as they underwent polarization.

The authors then extended their investigation beyond polarization and looked at the role of plastin in cytokinesis. In healthy zygotes, actin filaments, together with non-muscle myosin II, will accumulate in the middle of the dividing cells. From there they effectively pull

the membrane inwards so that opposing sides of the cell meet and fuse, thereby creating two cells. This did not occur at the same rate in cells containing mutated plastin, and it was determined that plastin facilitates the accumulation of the proteins in the correct position.

To better understand why plastin-mediated crosslinking of actomyosin filaments had these effects on cell polarization and cytokinesis, the team turned to mathematical modeling. Specifically, they tested whether increased connectivity between plastin and actomyosin alone is sufficient to drive long-range cortical contractility. These simulations revealed an optimal level of plastin crosslinking required to facilitate these processes, and indicated that too little or too much cross-linking will result in an F-actin network that is

either too disconnected or too stiff. In both cases, the end result is weakened contractility. To confirm that this was true in living organisms, the team increased the levels of plastin in the healthy *C. elegans* zygotes, and found that indeed, having too much plastin substantially slowed down cytokinesis.

Embryogenesis requires robust force generation and transmission that drives critical cellular processes such as polarization and cytokinesis. The findings presented in this work elucidate a role for plastin as a molecular rivet to facilitate robust polarization and timely cytokinesis. These discoveries further demonstrate the importance of the high-order organization of the actomyosin cytoskeleton and the role of actin binding proteins, such as plastin, in regulating its function.

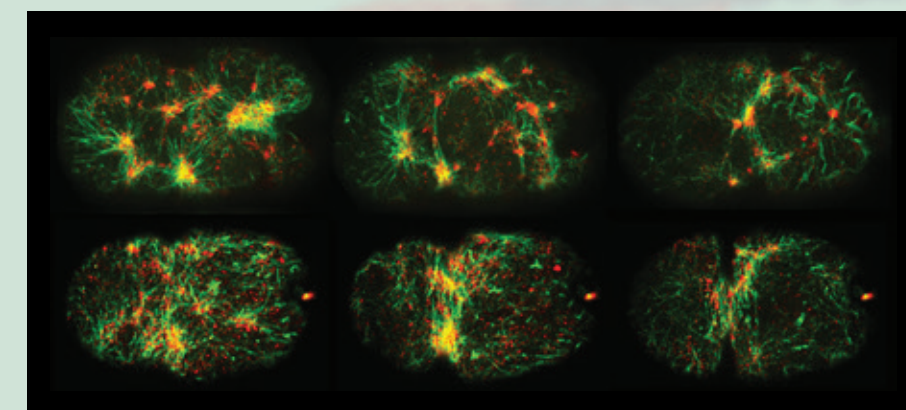


Figure: Plastin (green) and non-muscle myosin II (red) in the cortex of a newly fertilized *C. elegans* zygote during polarization (top panel) and cytokinesis (bottom panel), imaged using spinning disk confocal microscopy. Colocalization between green and red signals signifies localized cortical contractility.

ABOUT THE RESEARCHER: RONEN ZAIDEL-BAR



Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biomedical Engineering, NUS. The Zaidel-Bar

lab researches cell-cell and cell-matrix adhesion and the interplay of these forces with the actomyosin cytoskeleton.

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Ding Y et al, Plastin increases cortical connectivity to facilitate robust polarization and timely cytokinesis. J Cell Biol. 2017 May 1;216(5):1371-1386. doi:10.1083/jcb.201603070

ELONGATION BY CONTRACTION

PULLING FORCES DRIVE CHANGES IN CELL SHAPE

Written by Andrew Wong.

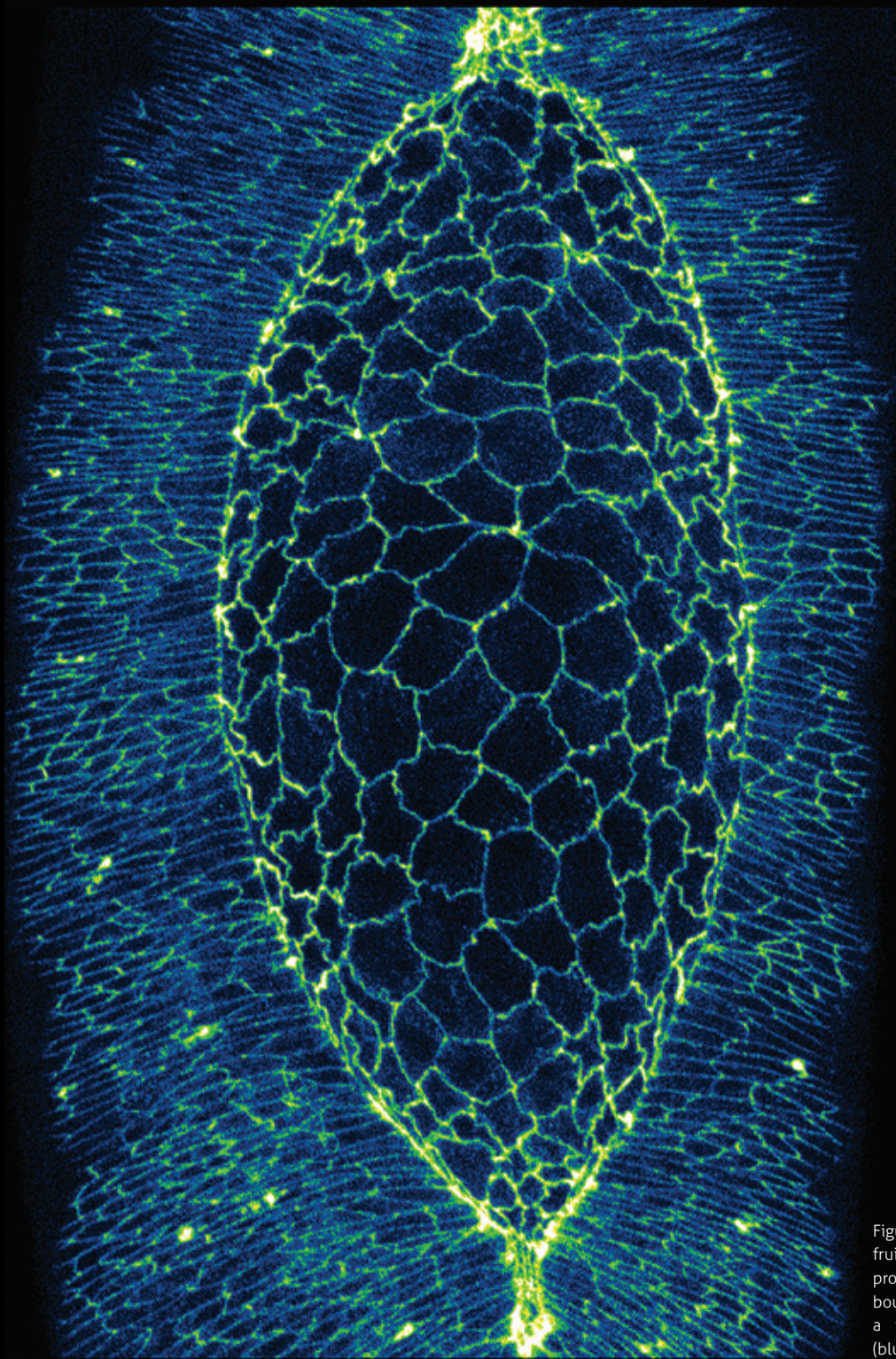


Figure: The amnioserosa of a fruit fly embryo undergoing the process of dorsal closure. Cell boundaries were labelled with a fluorescent protein marker (blue-green)

A team of scientists from MBI, National University of Singapore have discovered a new mechanism of cell boundary elongation. Elongation and contraction of the cell boundary is essential for directing changes in cell shape, which is required for the correct development of tissues and organs. This study is published in Current Biology.

How do contractile forces lengthen cell boundaries?

During development of an embryo, cells assemble to form tissues and organs. This requires cells to grow, divide, and occasionally undergo programmed death. However, a common property observed in many of these biological processes is cell deformation, which is essentially a dynamic change in cell shape. Based on mechanical principles, these shape changes have been broken down as either a contraction or elongation of the cell boundary.

Cell boundary contraction has been extensively studied, and is driven by contraction of a network of protein filaments within the cell. The two major components of this contractile network are actin, a structural protein that forms long filaments or cables, and myosin II, a motor protein that binds to actin and uses energy to slide the actin filaments past each other. Known as actomyosin, this network is responsible for generating contractile force in cells.

As actomyosin contraction is a "pulling" force, it is easy to visualize how activity of this network can pull in the cell boundaries, causing contraction. However, as the actomyosin network is unable to

generate the opposite "pushing" force, scientists have long sought to answer the question of how do cell boundaries elongate?

In order to answer this question, MBI researchers Dr Yusuke Hara and Murat Shagirov from the lab of MBI Principal Investigator Asst. Prof. Yusuke Toyama, used live embryo imaging and laser surgery to probe cell boundaries in the developing fruit fly embryo, a model system which has many similarities to mammalian development. The researchers focused their investigation on a patch of about 200 polygon shaped cells called the amnioserosa, which "zip up" to seal the embryo. The dynamic deformations that amnioserosa cells undergo makes them an ideal choice for studying cell shape changes.

Careful observation and computational analysis of the amnioserosa revealed that cells underwent rhythmic changes in both area (growing and shrinking in size), and also in boundary length (becoming longer and shorter). However, these two processes were not synchronized as might be expected - i.e. a cell increasing in area was not necessarily undergoing elongation of all of its boundaries. This complication prompted the scientists to examine the distribution of myosin II across the amnioserosa.

This led to the discovery that there was an increase in myosin II in the cells next to the cell undergoing boundary elongation. By disrupting the myosin II in these neighbouring cells with a laser, cell boundary elongation was terminated. This demonstrated that myosin II in neighbouring cells actively elongates the cell boundary via pulling of the boundary ends, much

like stretching a rubber band with your fingers.

This study revealed that actomyosin networks are responsible for both contraction and elongation of the cell boundary. Actomyosin contractility within a cell results in cell boundary contraction. However, it is the activity of the actomyosin network in surrounding cells that results in the elongation of the cell boundary, suggesting the cell boundary elongation in a tissue is not a single cell process, but one which requires help from neighbouring cells.

Understanding how actomyosin networks of neighbouring cells cooperate to direct changes in cell shape will be invaluable for deciphering many biological processes, such as tissue development, organ specialization, and wound healing.

ABOUT THE RESEARCHER: YUSUKE TOYAMA



Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biological Sciences, NUS. The Toyama lab uses interdisciplinary techniques, such as imaging, gene manipulation, and biophysical modelling to extract mechanical signatures from cell and tissue dynamics.

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REFERENCE:

Hara Y, Shagirov M, and Toyama Y. Cell boundary elongation by non-autonomous contractility in cell oscillation. Curr. Biol. 2016, Vol. 26, doi: 10.1016/j.cub.2016.07.003.

DECISIONS IN A PINCH

RECEPTOR TYROSINE KINASES CONTROL MECHANOSENSORS

Written by Steven Wolf and Sruthi Jagannathan. Illustration by Diego Pitta de Araujo.

Scientists from the Sheetz lab at the Mechanobiology Institute, National University of Singapore have identified a role of receptor tyrosine kinases in the regulation of the cellular mechanosensory machinery, which has relevance for understanding the basis of cancerous growth and developmental abnormalities. This work was published in Nano Letters.

Controlling the mechanosensory machines

Cells live in different micro-environments within the human body. Each of these micro-environments vary in their stiffness, from soft (like blood) to hard (like bone). Accordingly, cells have the ability to sense and react to different rigidities. For a tissue to develop properly, the cells within it must respond

correctly to various stimuli, including the information from their physical surroundings.

To measure the stiffness, or rigidity, of their surroundings, cells utilise nano-scale 'mechanosensory' machines. These machines work like human fingers. When they are 'pinching' on the matrix (various proteins and sugars that surround cells), they determine whether the immediate environment is soft, stiff, or even solid. This information will then tell the cell whether to move, or to stay and grow. To ensure a cell responds correctly to the information it senses, these nano-machines must be maintained under precise control by many 'on or off' regulatory switches.

When the 'on or off' signals are generated incorrectly, the cellular machinery will operate in an inappropriate manner,

and this can lead to the onset of various diseases. Cancer, in particular is associated with a loss of regulation, and the mis-control of mechanosensory, motility, and growth programmes. The ultimate result here, is that the cells ignore their micro-environment's rigidity, break free of their surrounding tissue, invade the bloodstream, establish themselves in otherwise foreign regions of the body, and begin to grow uncontrollably.

One class of protein that is critical in detecting signals and triggering an 'on or off' signal for other proteins are the Receptor Tyrosine Kinases (RTKs). Mutations or alterations in the RTKs are known to cause tissue malformation in development and they often function erratically in cancer. In fact, several successful cancer therapeutics target specific RTKs. Now,

MBI researchers have discovered that RTKs also regulate the ability of cells to test the rigidity or stiffness of their surroundings, by controlling the activity of mechanosensory machinery located at the cell periphery.

Led by MBI Director Prof. Michael Sheetz, the research team observed cells on microfabricated pillars using fluorescence microscopy and identified the formation of small contractile units, which resembled sarcomeres (the contractile units of muscle), forming at the front edge of the cell. Each sarcomere-like unit bridged two matrix attachment sites and pulled on them simultaneously, causing them to deflect inwards towards each other. When working properly, these pinching units tell the cell how stiff the matrix attachments are.

Knowing the importance of RTKs in the regulation of cell and tissue growth programmes, the team disrupted or removed various RTKs from these cells, and again observed how they used the sarcomere-like contractile units to pinch matrix beneath them. Confirming their suspicions, when one RTK, known as AXL

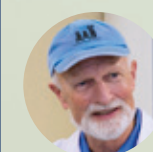
was disrupted, the cells pinched with greater force. However, when a separate RTK, known as ROR2 was disrupted, the pinching activity remained the same strength, but took place over a longer period of time. These alterations in the pinching machines caused them to respond to soft matrices as if they were rigid, which encouraged the cells to grow in soft environments, when they normally would not.

It is known that cancerous cells can ignore the rigidity of their surroundings and initiate cell motility or growth pathways regardless. Furthermore, altered interactions between AXL and components of the mechanosensory machinery had previously been attributed to the shift from normal cell growth to the establishment of malignant tumours, however the basis of this relationship remained unclear.

It is now believed that in some cancers, the ability of cells to spread around the body, and establish new tumours results from the erratic functioning of the mechanosensory machinery as a result of abnormal RTK signaling. This research renews the notion that targeting

specific RTKs is beneficial in the fight against cancer and it is hoped that with further research, scientists will identify new ways to regain the control of mechanosensory machines in cancerous cells to restore normal growth patterns.

ABOUT THE RESEARCHER: MICHAEL SHEETZ



Director of the Mechanobiology Institute, Distinguished Professor of the Department of Biological Sciences, NUS, and Emeritus Professor of the Department of Biological Sciences at Columbia University, USA. The Sheetz lab focuses on understanding the molecular mechanisms of mechanotransduction and their involvement in a variety of phenomena from cancer metastasis to brain function.

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REFERENCE:

Bo Y et al, Mechanosensing Controlled Directly by Receptor Tyrosine Kinases, Nano Letters DOI: 10.1021/acs.nanolett.6b02995.

MICROSCOPIC MUSCLES

HOW NON-MUSCLE CELLS FIND THE STRENGTH TO MOVE

Written by Steven Wolf. Illustration by Diego Pitta de Araujo.

ABOUT THE RESEARCHER:

ALEXANDER BERSHADSKY



Principal Investigator at the Mechanobiology Institute, and the Joseph Moss Chair Professor of Biomedical Research, Weizmann Institute of Science. The Bershadsky lab research interests include the mechanisms of cell motility and intracellular traffic, cytoskeleton dynamics, and the crosstalk between the cytoskeleton and cell adhesion.

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REFERENCE

Hu S et al. Long-range self-organization of cytoskeletal myosin II filament stacks. *Nature Cell Biology*, 19, 133–141 (2017) doi:10.1038/ncb3466

Through the use of advanced imaging, researchers from MBI, National University of Singapore have described, for the first time, the ordered arrangement of myosin-II filaments in actin cables of non-muscle cells. This work is published in *Nature Cell Biology*.

Defining the ordered arrangement of myosin-II filaments in non-muscle cells

The twitching contractions of our muscle cells are well known. They can be detected just weeks after conception as the embryonic heart begins beating. Muscle cell contractility is produced from interactions between protein-based cables of the cytoskeleton and small molecular motor proteins known as myosins.

There are over 200 types of cells within the human body, and not all need to repeatedly contract. Despite their distinct functions, nearly all cells contain the same basic protein components found in muscle cells. Importantly, most cells also exhibit some degree of slow contractility. Fibroblasts are one such example. Found in connective tissue, these cells produce the material that surrounds all cells, and ultimately defines tissue shape. Importantly, fibroblasts are also known to remodel this material, and for this, they need strength to pull against their environment.

To investigate the organization of the cytoskeleton and its associated motor proteins in non-muscle cells, researchers from the Mechanobiology Institute, National University of Singapore, analyzed fibroblasts using a form of super resolution microscopy known as Structured Illumination Microscopy (SIM). The researchers, who were led by Prof. Alexander Bershadsky and Asst. Prof. Ronen Zaidel-Bar, focused their investigation on the assembly of the cytoskeleton. Along with providing

structural support to the cell, the cytoskeleton can also buffer stresses from the external microenvironment and give cells the power to contract and move through a tissue. These processes are possible due to the continual assembly and disassembly of the protein cables, and due to the generation of force as motor proteins pull on these cables.

When the cytoskeleton was viewed in living fibroblasts, Dr Shiqiong Hu, a postdoctoral researcher at MBI, and colleagues, discovered unique, organized patterns of motor protein filaments within large protein cable-like structures known as stress-fibers. These cables form dynamically and often bridge sites where the cells are interacting with the microenvironment.

Like ropes, these cables are made up of many individual filaments, held together by various cross-linking proteins. By watching the cytoskeleton form over time, the researchers observed how myosin-II filaments arranged into stacks that ran perpendicular to the large parallel stress fibers. These stacks alternated with regions of the 'cross-linking' protein α -actinin, which tethers individual filaments together to produce the protein cable.

How myosin-II filaments come to be stacked together within the bundled stress fibers, remains to be fully defined, however one observation from this study that may hold the answer, is the long range movement of myosin-II filaments towards each other. As the researchers propose, this attraction may result from contractile or elastic forces generated by the myosin filament stacks, which can transmit through the surrounding cytosol to individual filaments that are otherwise isolated.

The stacking of myosin-II filaments in non-muscle cells like the fibroblast is an intriguing element in the self-organization of the cytoskeleton, and overall architecture of the cell. Fibroblast function requires the cell to

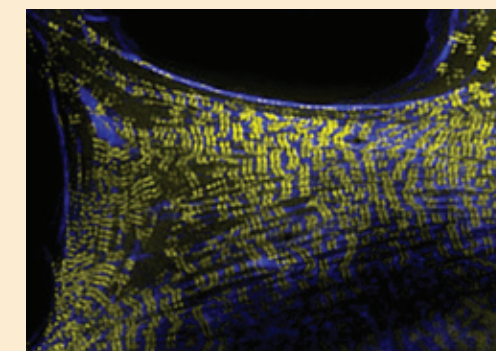


Figure: Visualization of myosin II filament stacks (yellow) with cross-linking protein α -actinin-1 (blue) in a rat embryo fibroblast (REF52) cells using structured illumination microscopy (SIM, Nikon). Myosin II filaments alternate with α -actinin enriched domains along actin stress fibers.

be able to stretch, generate cytoskeletal protrusions and move to other regions of the connective tissue. The assembly and organization of myosin-II into stacks permits the fibroblast to fulfill these cellular processes.

Even in non-muscle cells the architecture of the cytoskeleton is specialized for force generation and sensing. The organization of the cytoskeleton in non-muscle cells is strikingly similar to that in muscle cells. In both cases contractile and elastic forces are integral in establishing a functional cytoskeleton, and once formed, a pattern of repeating protein-based contractile proteins becomes evident. However, unlike in muscle cells, these structures continuously assemble and disassemble in non-muscle cells, allowing them to adapt their function, shape, and direction of movement according to the environment they find themselves in.

As observed in this study, even non-muscle cells require the strength to pull against their surroundings, and fight their way through often sticky environments. This strength comes from a highly refined system of filaments and motor proteins. Although not as strong as those found in muscle cells, their organization in non-muscle cells allows them to remain responsive to changes in the environment, whilst providing just the right amount of force to carry out their functions.

SIGNALING IN 3D

Written by Lakshmi Ramachandran. Illustration by Melanie Lee.

ADDING A SPATIAL DIMENSION TO CELL SIGNALING

A collaborative study from MBI, National University of Singapore, and the FIRC Institute of Molecular Oncology (IFOM), Italy, has shown for the first time, how the shape of cells within tissues determines their response to biochemical signals. This study, led by Aninda Mitra, IFOM postdoctoral fellow, together with Saradha Venkatachalapathy, MBI graduate student from the lab of Professor GV Shivashankar, Deputy Director of MBI, demonstrates the differential impact of two divergent cell shapes/geometries on gene regulation by TNF α , an immune regulatory protein that plays a role in cancer. This work is published in PNAS.

How does cell geometry regulate biochemical signaling?

The cells that make up the human body are constantly responding to, and generating, biochemical and mechanical signals. These signals include hormones, growth factors, neurotransmitters, immune

regulators (cytokines), as well as sensory signals. Whether these signals originate from mechanical (blood, pressure, or touch) or biochemical sources, they are nearly all transmitted via a series of biochemical reactions that converge on the cell nucleus. With the nucleus housing the cell's genome, any signal that reaches it will impact which genes are turned on or off. This in turn initiates distinct cellular responses. The ability to specifically activate or deactivate genes is crucial for normal cell physiology and function, and any aberrations in cell signaling may lead to disease states such as fibrosis and cancer.

Years of research into biochemical signaling has already provided researchers with a strong understanding of how gene programs are defined by specific biochemical pathways. Recent studies have also shown that mechanical properties of the extracellular matrix (ECM), which is a complex mesh of fibrous proteins and polysaccharides on which cells embed and attach, also regulate cell behavior. Despite the vast range of biochemical and mechanical signals already defined, it is becoming increasingly

clear that cells also take on important information from external sources such as their immediate microenvironment.

With this in mind, a team of researchers from the FIRC Institute of Molecular Oncology, who are based at the Mechanobiology Institute, Singapore, sought to determine whether physical properties that define the cell as a whole, can influence the biochemical pathways that drive cell function.

One property that was of particular interest to the team was cell geometry. That is, the shape that cells assume as they are organized into various tissues. Importantly, changes to cell shape are commonly observed in cancer. Cells found in healthy connective tissue, for example, have an elongated shape, yet these same cells become round as they turn cancerous. The rounding up of cancer cells is also associated with their detachment from the ECM and neighbouring cells.

It had recently been shown that cells can exhibit distinct gene expression patterns if their geometries were altered. This inspired the researchers

to assess the influence of cell geometry on a biochemical signaling pathway that is commonly impaired in cancer; the tumour necrosis factor alpha (TNF α) pathway. To achieve this Mitra et al used techniques to grow cells in the lab that mimicked two naturally occurring cell geometries - a rectangular, stretched shape, and a circular relaxed shape. The cells were then stimulated by adding the cytokine TNF α .

What was discovered was that the pattern of genes switched on or off was significantly different depending on whether the cell geometry was rectangular or circular. Importantly, the scientists demonstrated that these genetic differences are related to the varying states of cytoskeleton organization in the two cell geometries. The cytoskeleton is essentially a network of protein cables that provide structural support to the cell. These same filaments can also be assembled into structures that allow the cells to interact with both the surrounding matrix, and neighboring cells.

In rectangular cells, the cytoskeleton is robust and well-organized, and this is in

contrast to circular cells. This structural disparity alone was sufficient for proteins that are controlled by TNF α to move into the nucleus of the rectangular cells, and bring about diverse patterns of gene expression.

The finding that the mechanical states of cells helps dictate their response to biochemical signals has far-reaching implications. By taking into account the three-dimensional organization of cells

in tissues, this study closely mimics the situation cells naturally exist in, to clearly demonstrate how the TNF α biochemical signaling pathway is regulated by cell geometry. In this case, biochemical and mechanosignaling pathways are mutually inclusive. These fresh insights into how the altered mechanical states affects genome regulation in tumors also shed light on the pathophysiology of cancer, and open up new avenues to investigate the cause of this disease.

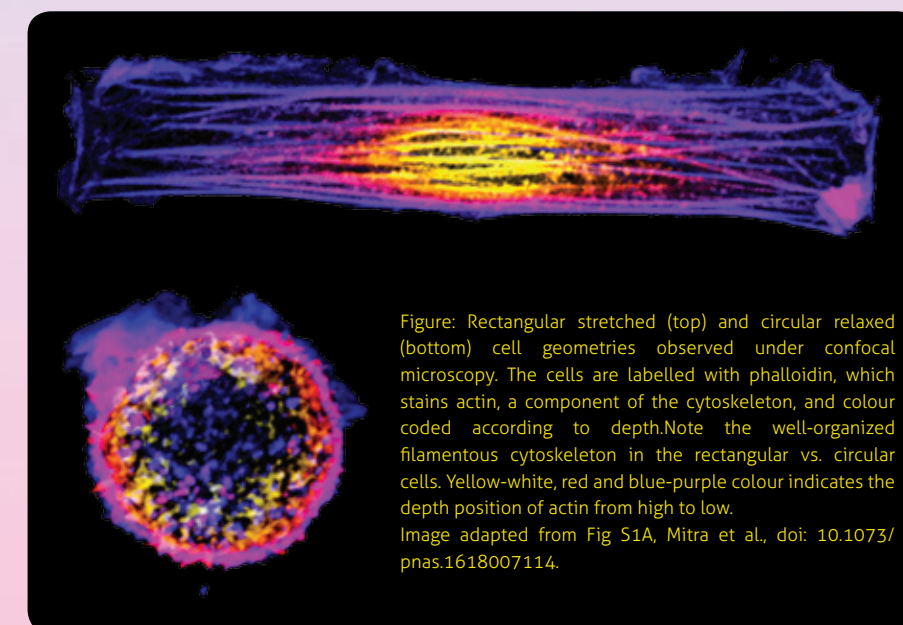


Figure: Rectangular stretched (top) and circular relaxed (bottom) cell geometries observed under confocal microscopy. The cells are labelled with phalloidin, which stains actin, a component of the cytoskeleton, and colour coded according to depth. Note the well-organized filamentous cytoskeleton in the rectangular vs. circular cells. Yellow-white, red and blue-purple colour indicates the depth position of actin from high to low. Image adapted from Fig S1A, Mitra et al., doi: 10.1073/pnas.1618007114.

ABOUT THE RESEARCHER: G.V. SHIVASHANKAR



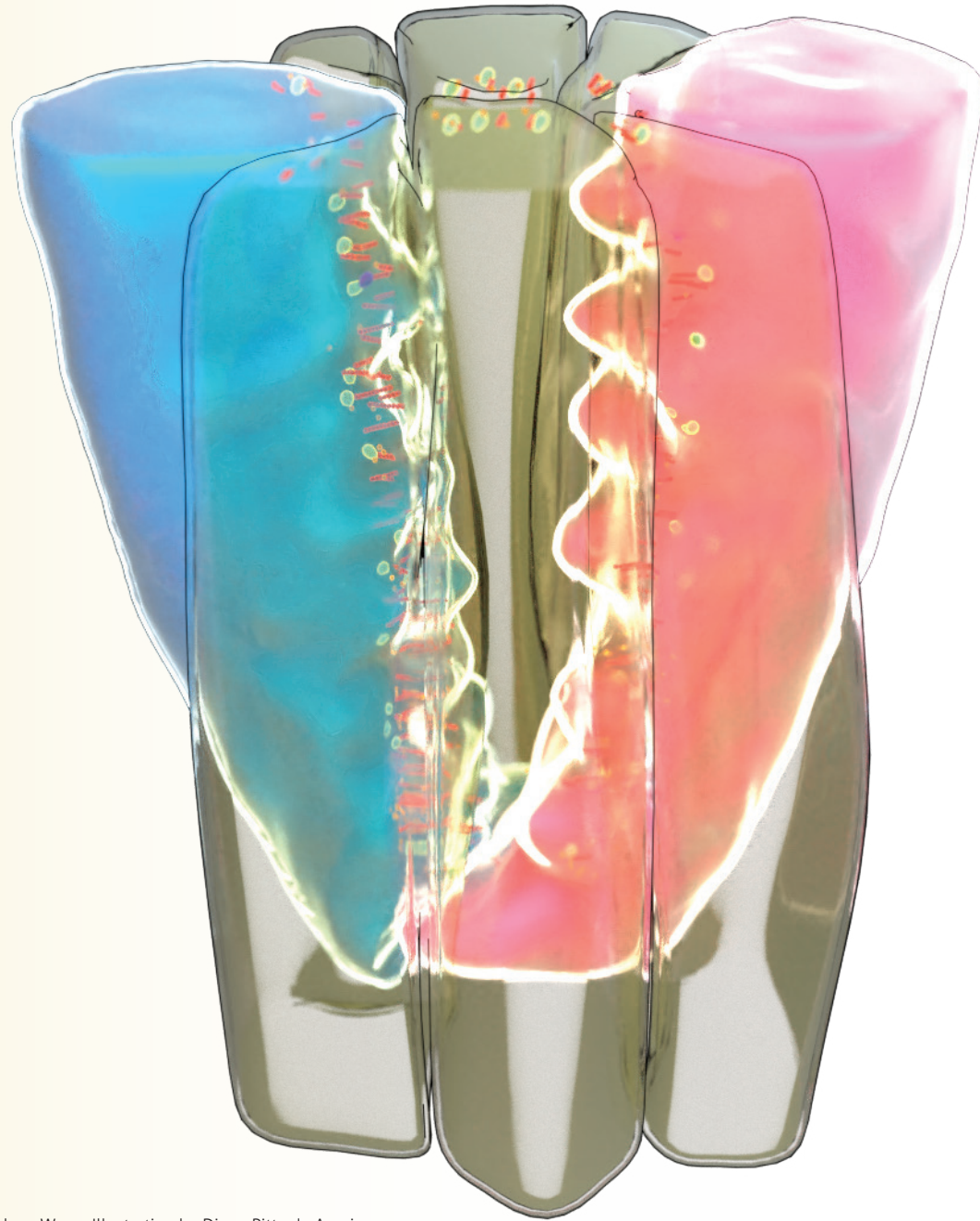
Deputy Director of the Mechanobiology Institute, Associate Professor at the Department of Biological Sciences, NUS, and IFOM-NUS Chair Professor. The Shivashankar lab pursues research in understanding the role of cell geometry on nuclear mechanics and genome regulation.

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FROM THE BOTTOM UP



Written by Andrew Wong. Illustration by Diego Pitta de Araujo.

ABOUT THE RESEARCHER: YUSUKE TOYAMA



Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biological Sciences, NUS. The Toyama

lab uses interdisciplinary techniques, such as imaging, gene manipulation, and biophysical modeling to extract mechanical signatures from cell and tissue dynamics.

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For more than a decade, the coordinated movement and rearrangement of cells during fruit fly embryonic development was thought to be controlled by contractile activity at the top of the cells, followed by the passive movement of the rest of the cell, including the base. However, scientists from the Mechanobiology Institute, National University of Singapore have discovered that protrusions extending at the bottom of the cell actually precede the movements at the top, and these basal protrusions play significant roles in directing tissue elongation.

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Figure: Artistic illustration of rosette formation. Protrusions extending from the bottom of dorsal (blue) and ventral (red) cells form a rosette at the base of the cell, while contractile activity between anterior/posterior cells (yellow) form a rosette at the top of the cell.

Uncovering the secret dynamics at the base of the cell

As embryos develop into an adult organism, they have to undergo numerous changes in shape and size. Sometimes this can be as a result of cell division and growth, but other times it is dependent on cellular rearrangement and coordinated collective cell movements.

One of the ways that cells undergo tissue elongation is by cell intercalation. This is the process by which neighbouring cells move and insert in between one another to rearrange their organization. This enables changes in the shape of the tissue, typically without a change in the overall number of cells.

Often, cell intercalation leads to spreading of tissue along one axis, at the expense of narrowing along the perpendicular axis. An example of this occurs during fruit fly embryo development by a process known as germ-band extension. During this developmental stage, the germ-band tissue shrinks along the stomach-to-back axis, and extends to nearly double in length along the head-to-tail axis.

How does this cellular rearrangement take place? To visualize this, imagine looking down at a line of cells arranged vertically, from north-to-south. During germ-band extension, these cells need to rearrange horizontally along an east-to-west orientation. If this line was a queue of people, the simple solution would be for each person to leave the vertical line and rearrange themselves individually in a horizontal line.

However, if this happened in a tissue it would require the cells to detach from one another, which would create gaps in the cell sheet and a damaging loss of tissue integrity. Cells remain connected to each other through cell adhesions,

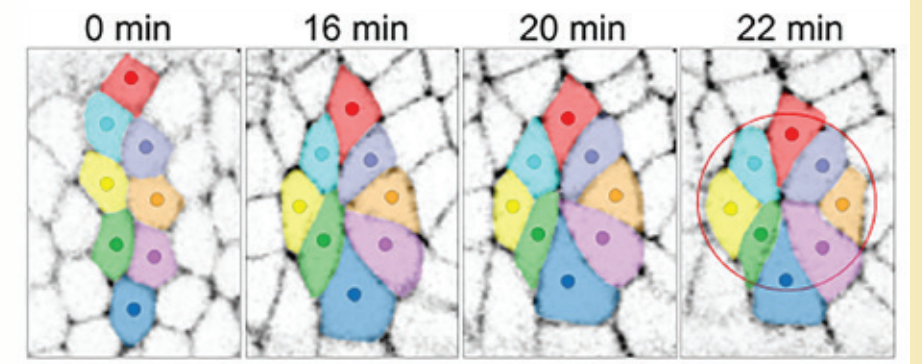


Figure: Apical rosette formation (red circle) during cell intercalation. Cells are colour-coded to illustrate movement and rearrangement. Adapted from Sun et al., doi: 10.1038/ncb3497

protein complexes located near the top of the cell.

Fortunately, these cell adhesions are dynamic, so during cell intercalation the cells first rearrange into a flower-like 'rosette' pattern, leading to a decrease in the vertical axis, and from this rosette the cells then elongate in the horizontal axis.

In the case of germ-band extension, years of scientific study has led to the general understanding that this dynamic remodeling of cell adhesions by contractile force is the key mechanism driving cell intercalation. The formation of an apical rosette near the top of the cell is a hallmark of this process.

Surprisingly, given that cells are three-dimensional (3D), very little was known about cell dynamics at the bottom of the cell during rosette formation. This shortcoming was investigated by MBI Principal Investigator Asst. Prof. Yusuke Toyama, his graduate student Zijun Sun, and an interdisciplinary team of MBI scientists. Using advanced live imaging of germ-band extension in fruit fly embryos, coupled with 3D reconstruction, the research team were able to observe in detail the changes in shape from the top to the bottom that the cell undergoes during intercalation.

They discovered that a rosette pattern also formed at the base of the cell, and remarkably, these basal rosettes formed earlier than apical rosettes. Unlike apical

rosettes, the formation of basal rosettes are not driven by contractile fibers, but instead by wedge-shaped protrusions that extended from the base of the cells, which then migrate towards each other during intercalation. Interfering with the formation of the basal rosettes leads to delayed germ-band extension, demonstrating that these basal rosettes are not merely a byproduct of the apical rosette, but are in fact active in cell intercalation. While basal and apical rosettes could form independently of one another, the cooperative activity of both rosettes is required for normal cell intercalation and germ-band extension.

By observing the cell in 3D, this study has uncovered a new perspective on cell intercalation. Previously, this process was thought to be solely directed by contractile activity at the top of the cell. However, this study turns our understanding of cell intercalation upside down, by clearly demonstrating that the first steps actually start at the base of the cell, where protrusive migration drives formation of basal rosettes. Understanding how cell intercalation depends on cellular movements ranging from the bottom to the top of the cell will provide fresh insight into embryonic development, and may help our knowledge of cancer, where the invasive, metastatic activity of tumour cells has many similarities with cell intercalation. Above all, this study shows how important it is to consider the other side of the coin - An unexpected fact might be waiting to be found...

THE (BP)GAP BETWEEN SIGNALING PATHWAYS

TO GROW OR NOT TO GROW?

Written by Sruthi Jagannathan. Illustration by Melanie Lee.



Researchers from the Mechanobiology Institute, National University of Singapore, under the guidance of MBI Principal Investigator Assoc. Prof. Low Boon Chuan highlight the role of BPGAP1 in linking major signaling pathways during cell proliferation. This study is published in *Oncogene*.

Ever wondered about the inner workings of the human body? Think of the body as a single, macro-entity, and chances are that you may still not be amazed by this phenomenal system. But, when you start imagining it as an ensemble of trillions of cells that are constantly talking to each other and passing on crucial information about each and every function that takes place inside, you would be, without any doubt, astounded by the complexity of this living machine.

So how do cells talk to each other? By sending out messages in the form of chemical molecules. When these chemical messages reach the receiving cell, they trigger a chain reaction in which one protein switches on or off a second protein, and the second protein switches on or off a third protein, and so on. In this way the message is carried through a series of proteins until it finally reaches inside the nucleus.

The nucleus, which is the information center of the cell, processes the incoming message and scales up or down the production of certain proteins. Such chain reactions are known as signaling pathways, and based on the type of proteins that are scaled up or down, cells decide what they should do next- change shape, grow, divide, move or die.

The signaling short circuit

For instance, signaling pathways are able to directly control how cells divide and grow in numbers. The process, known as cell proliferation, is commonly observed in embryos where cells proliferate to form tissues and organs or during adult life when there is a need

to replenish cells that are lost due to damage. However, problems arise when signaling pathways lose their control over this fundamental cellular function. Similar to how short circuits in electrical connections may trip power supplies, so can any short circuits in signaling pathways lead to 'tripping' of cellular functions, causing the cells to behave abnormally. In this case, cells may start dividing frantically and form lumps of cancerous tissue that can interfere with normal body functions and often prove to be fatal.

Two pathways – JNK and MEK pathways (named after proteins that have important roles in the two pathways) – are well-known to influence the way cells grow and divide. Not surprisingly, short circuits in these pathways have been associated with a number of cancer types. Although a number of studies have provided evidence that JNK and MEK pathways work together to produce effects on cells, it is not very clear how the two pathways are connected during cell proliferation.

Due to their importance in cell proliferation and cancer, a team of researchers at the Mechanobiology Institute were keen to find out how the two pathways are connected in cells. By carrying out a number of protein-based tests, they found that the JNK and MEK pathways act together as a larger signaling network.

How the signaling network operates

When chemical messages responsible for growth and proliferation reaches cells, the JNK pathway is triggered as the first level of response. A protein belonging to the JNK pathway, called JNK1, then switches on another protein called BPGAP1 by adding chemical compounds (more specifically, a phosphate molecule) to BPGAP1. The BPGAP1, in its 'on' state, recruits two more proteins- MP1 and MEK1- and facilitates interaction between these

two proteins. MEK1 belongs to the MEK pathway, and the interaction between MP1 and MEK1 switches on several other proteins in the MEK pathway. The end point of this signaling network is the protein Erk moving into the nucleus, where it affects the production of proteins responsible for cell growth and division.

The study identifies BPGAP1 as the protein that is mainly responsible for linking two major signaling pathways controlling cell growth and proliferation. By being linked, the two pathways are able to talk to each other and maintain a balance in cell numbers. On the flip side, any short circuit in one pathway is easily communicated to the other pathway; proteins in both pathways are erratically switched on that leads to uncontrolled growth and proliferation of cells into tumors. This is evident from a number of studies that show a higher risk for cancer development and recurrence as a result of short circuits in the JNK-MEK signaling network.

By bringing out the cancer-causing potential of BPGAP1, the study creates avenues for its possible use as a therapeutic target in future cancer studies.

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PERSISTENT α -CATENIN ACTIVATION

Written by Lakshmi Ramachandran. Illustrations by Melanie Lee, Diego Pitta de Araujo, and Chun Xi Wong.

Members of the Groves Lab at MBI have discovered that α -catenin, once activated by forces applied during the process of E-cadherin clustering, remains activated irrespective of the presence of continued force. This new finding challenges the previous notion that activated α -catenin is force-sensitive. This study was led by MBI Senior Research Fellow Dr. Kabir H Biswas, from the lab of Prof. Jay T. Groves, and published in the Biophysical Journal.

Sticky proteins in cells

Cells remain glued to each other in tissues through adhesive structures formed by dedicated proteins at cell-cell junctions. These Adherens Junctions (AJ) are highly dynamic, meaning that they can assemble and disassemble in response to various physical cues from the environment. The major component of AJs is E-cadherin, a protein that spans the cell membrane. The inner regions of E-cadherin interact with the structural proteins of the cell, mainly the actin cytoskeleton, while the outer regions connect to an E-cadherin on an adjacent cell.

catenins join in to link E-cadherin with the actin cytoskeleton, which strengthens and stabilizes the AJ.

Force-locked activation

α -catenin is one of the three catenins found at AJs and is thought to function as a transducer of mechanical forces. Previous work has shown that α -catenin at E-cadherin adhesions undergoes force dependent activation from a 'closed' to an 'open' conformation. It is the open, activated form of α -catenin that binds actin. However, the precise mechanics of α -catenin activation was not known due to limitations in experimental systems. For example, monolayer cell cultures are often used to study cell-cell junctions, but in this live cell system it is difficult to control the dynamics of E-cadherin clusters or perturb E-cadherin cluster assembly.

In order to overcome these limitations, Biswas et al. studied adhesion formation between a live cell and an artificial membrane functionalized with E-cadherin that mimics the cell membrane of the adjacent cell. This system is dubbed the hybrid live cell-supported lipid bilayer (SLB) platform and offers control over both the formation as well as inhibition of E-cadherin cluster assembly. E-cadherin cluster formation can be optimized on SLBs by controlling the density of E-cadherin and consequently their mobility, whereas E-cadherin assembly can be perturbed by using tiny, nano-scale grids on SLBs that physically block E-cadherin movement.

The process of AJ formation in epithelial cells begins with the extension of finger-like exploratory projections, called filopodia, from the surface of adjacent cells. Protrusion and retraction of filopodia results in the formation of micro-clusters of E-cadherin at the cell-cell interface. Following this initiation of adhesion, additional proteins called

were found to associate with these clusters. However, activated α -catenin additionally associated with E-cadherin clusters located in the centre of the interface, which were devoid of cytoskeletal proteins or force. Based on this the researchers noted that although E-cadherin cluster formation is essential for the initial activation of α -catenin, neither association with actin cytoskeleton, nor cellular forces, is required for the persistent activation of α -catenin.

This study provides new insights into mechanical signal transduction at adherens junctions. First, the study reveals a previously unknown mechanism of α -catenin activation, which is the requirement of E-cadherin clustering to initiate its activation. Second, the observation from this study that activated α -catenin is not sensitive to force, challenges the previous notion that α -catenin may function as a force-transducer at E-cadherin adhesions. This large pool of activated α -catenin that is not associated with actin filaments may be held in reserve by the cell in the event of an increase in intracellular tension.

ABOUT THE RESEARCHER: KABIR H BISWAS



Senior Research Fellow in the lab of Prof. Jay Groves at the Mechanobiology Institute. He is interested in understanding mechanisms of cellular signal transduction mediated by cell membrane localized as well as cytosolic proteins

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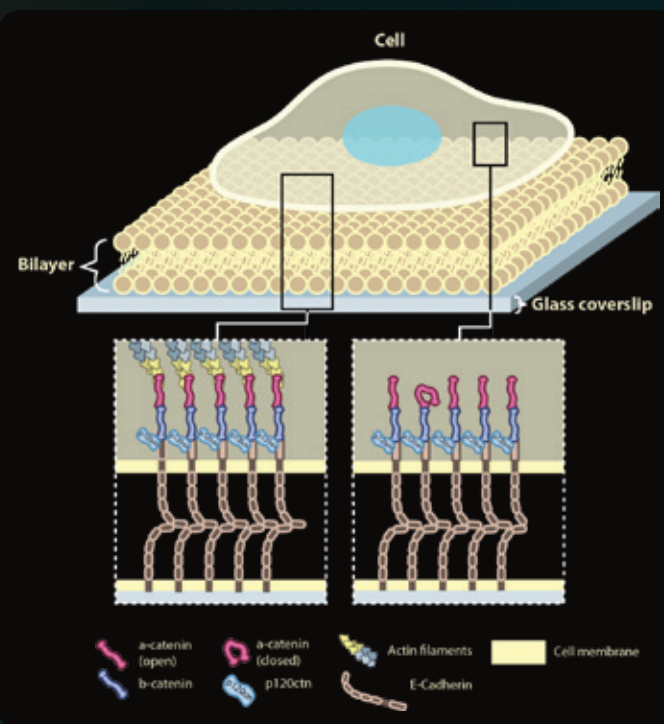


Figure: A schematic showing a cell making adhesive contacts with the supported lipid bilayer. Left inset shows peripheral E-cadherin clusters interacting with activated α -catenins and the actin cytoskeleton. Right inset shows central E-cadherin clusters with α -catenins, the majority of which are activated despite the absence of cytoskeletal contacts.

STABILITY WITHOUT JUNCTIONS

Written by Andrew Wong.

Scientists from the Mechanobiology Institute, National University of Singapore, have discovered that cadherin clusters, which are well known for forming junctions between cells, also play a role in stabilizing the cell cortex. This study was published in Current Biology.

A new role for cadherin

Multicellular life depends on the ability of cells to adhere to one another. This takes place through cell-cell junctions, protein complexes that physically connect cells together. At the core of cell-cell junctions is the protein cadherin, which spans across the cell membrane, sticking out of the cell to connect to cadherins on neighboring cells. Cadherin also attaches to the internal cell cortex, a dense layer of proteins underneath the cell membrane which has two major components: the filament-forming protein actin that provides structural stability, and the motor protein myosin that enables dynamic movement of the cortex depending on the needs of the cell. This physical bridge between cells enables the transmission of both mechanical and biochemical signals across multicellular tissues.

ABOUT THE RESEARCHER: RONEN ZAIDEL-BAR



Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biomedical Engineering, NUS. The Zaidel-Bar lab researches cell-cell and cell-matrix adhesion and the interplay of these forces with the actomyosin cytoskeleton.

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Padmanabhan et al, Non-junctional E-Cadherin Clusters Regulate the Actomyosin Cortex in the *C. elegans* Zygote, Current Biology, doi: 10.1016/j.cub.2016.10.032).

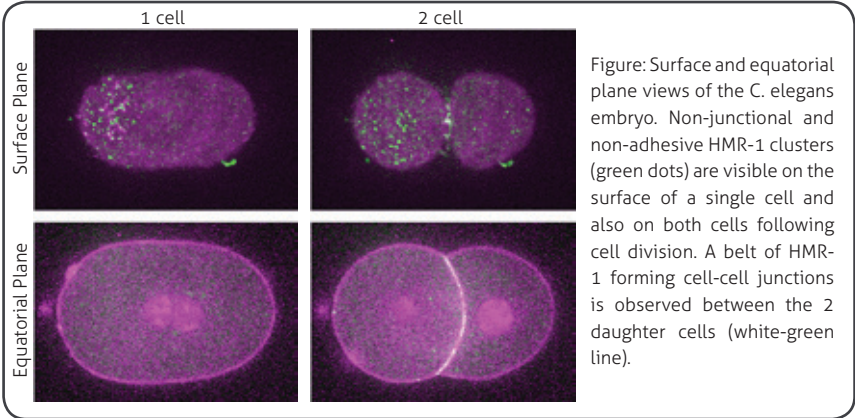


Figure: Surface and equatorial plane views of the *C. elegans* embryo. Non-junctional and non-adhesive HMR-1 clusters (green dots) are visible on the surface of a single cell and also on both cells following cell division. A belt of HMR-1 forming cell-cell junctions is observed between the 2 daughter cells (white-green line).

However, scientists have observed clusters of cadherin on the cell surface which are not involved in cell-cell junctions. While it has been speculated that these non-junctional and non-adhesive cadherin clusters are being kept in reserve in order to strengthen or create new cell-cell junctions, the actual function of these clusters remained unknown.

With their expertise in cell adhesion and developmental biology, MBI Principal Investigator Asst. Prof. Ronen Zaidel-Bar and research fellow Dr. Anup Padmanabhan used embryos from the nematode *C. elegans* to probe the function of these non-junctional cadherin clusters. After tagging the worm equivalent of cadherin, a protein named HMR-1, with a fluorescent marker, they were able to follow its location and movement by live imaging. Focusing their investigation on the zygote, the single fertilized egg cell that develops into an embryo, they discovered that HMR-1 formed non-junctional, non-adhesive clusters similar to cadherin. Even though these non-junctional HMR-1 clusters did not form connections outside of the cell, they still remained internally associated with actin filaments of the cell cortex, but not the myosin motor proteins. In fact, the presence of non-junctional HMR-1 clusters prevents cortical accumulation of myosin and decreases the contractile activity of proteins that drive cortical movement,

In order to determine whether non-junctional HMR-1 affected cytokinesis – the physical process by which the cell cortex rotates

and contracts to divide the cell into two – the scientists genetically altered the level of HMR-1. Reducing the amount of HMR-1 resulted in faster cytokinesis while increasing HMR-1 levels slowed it down, demonstrating that these non-junctional clusters have a key function in regulating movement of the cell cortex. Analysis of cortical dynamics during cell division revealed that HMR-1 clusters attached to the actin filaments effectively provided drag against cytoskeleton movement, by acting as structural anchors lodged in the cell membrane. The importance of this anchoring in maintaining cell integrity became clear following extended observation of embryos with reduced levels of HMR-1, which were vulnerable to cortical splitting, where a segment of cortex tears away from the cell membrane.

In essence, the non-junctional HMR-1 clusters can be thought of as cellular staples that help secure the cortex to the cell surface. The friction from the clusters stabilizes the cortex and slows down cortical flow, preventing dramatic cortical deformation, while allowing enough cortical movement for fundamental processes like cytokinesis. This new discovery means that scientists must re-evaluate their understanding of cadherin. The importance of non-junctional cadherin in stabilizing the cell cortex must now be considered along with the classical function of cadherin in maintaining cell-cell junctions. This fresh perspective may unlock new avenues of investigation regarding the role of cadherin in health and disease.

LETTING GO

Written by Steven Wolf.

Figure: The purse-string (red oval) contracts to detach the dying cell from its neighbours

In a study published in Development, scientists from MBI, National University of Singapore have described how dying cells detach and are expelled from a tissue, and how tissue tension in the region surrounding a dying cell is remodeled.

How dying cells detach from their neighbours

The tissues in our bodies, such as epithelial, nervous or muscle tissue, are all made up of millions of cells that are tightly packed together. Each cell lives in these conditions as an individual unit, but with their primary function dictated by the overall role of the tissue. Epithelial tissue, is found on the surface of organs or in the bodies cavities, and provides a means of protection; preventing fluids and other nutrients from leaking out of the body, and stopping harmful toxins from entering.

The integrity of any tissue is crucial for their function, and this is especially the case with epithelial tissue. Just as a plastic bag would leak if it was punctured, so too would epithelial tissue if cells are damaged through injury or disease. Cells are also prone to dying when they are damaged and even the smallest holes left when a single cell dies, must be filled immediately in order to maintain the integrity of the tissue. The dying cell however, needs to be detached from its neighbours, and expelled, in a process known as cell extrusion. If this does not occur, the dying cell is stacked in the tissue and causes further damage to the neighbouring healthy tissue. Removing a dying cell from a tissue without compromising the tissue integrity is therefore, not a trivial process.

To understand how dying cells are removed from epithelial tissue while the surrounding tissue remains intact and functional, researchers from the Mechanobiology

Institute, led by Asst. Prof. Yusuke Toyama, furthered their earlier investigations by viewing cells in a developing fruit fly pupae using live imaging.

By analyzing properties of cells surrounding a dying cell, as well as the dying cell itself, the team revealed a carefully choreographed sequence of molecular events that revolved around an interplay between the molecular structures holding the cells together, and the protein based contractile cables in cells located in the immediate area.

Firstly, the proteins that connects the dying cell to its neighbours begin to be remodeled. Normally these adhesive structures exist in between cells of a tissue to physically hold the cells together. They also provide a means of communication between the cells. As the adhesions are remodeled and the protein components that make up the adhesion are reduced, the cells surrounding the dying cell essentially let it go. The result is a temporary reduction in tissue tension that extends into the tissue, away from the dying cell. This disengagement of cell adhesions allows the cell to be released from the tissue. However, to prevent the tissue from becoming leaky, the hole left behind must be closed immediately.

This process was found to involve the formation and contraction of a protein based cable that passes through each cell surrounding the hole. Although this “purse-string contraction” has been described previously, the authors discovered that its formation was directly linked with the reduction in cell-cell adhesion components, and the subsequent detachment of the dying cell from its neighbours.

In this case it is the release of tension that was proposed to be the event that triggers the accumulation of the proteins required to

make the contractile cable. The contraction of the cable then rebuilds tissue tension, which forces the cells that surround the hole to become stretched. Stretching the cells brings them within proximity of the cells on the other side of the hole, with which they can form new cell-cell contacts. This eventually reveals the gap that transiently formed to let the dying cell go.

Ultimately, these findings provide new insights into the dynamic cellular processes that occur when tissue integrity is compromised. To avoid harmful consequences the body initiates a sequence of events that, as this study shows, is highly co-ordinated, and dependent on the physical interactions occurring at the cellular level. With tissue engineering reliant on our better understanding of how our bodies naturally form and maintain healthy tissue, the findings from this study will help scientists better implement engineered tissues in the future.

ABOUT THE RESEARCHER: YUSUKE TOYAMA



Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biological Sciences, NUS. The Toyama lab uses interdisciplinary techniques, such as imaging, gene manipulation, and biophysical modeling to extract mechanical signatures from cell and tissue dynamics.

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ILLUMINATING THE CONTACTS

USING SUPERRESOLUTION IMAGING TO MAP ADHERENS JUNCTION MACHINERY

Written by Steven Wolf and Sruthi Jagannathan. Illustration by Diego Pitta de Araujo.

The development of super resolution microscopy has revolutionized how scientists view and understand the inner workings of the cell. Just as advances in satellite camera technology gave rise to highly detailed maps of the world, so too has super-resolution microscopy allowed researchers to build detailed maps of individual cells. Such is the detail, that not only is the location of individual protein-based machines achievable, but these machines can be broken down into their parts, and the position and orientation of these parts, mapped out as well.

In the human body, cells rarely function in isolation. Instead they exist as part of multicellular communities that make up tissues and organs. To ensure the tissue functions correctly, individual cells must remain in physical contact with their surrounding cells. When cells are unable to maintain this contact, devastating diseases may arise, cancer being one of the most dreaded examples.

Cell-cell adhesion sites are found at specific regions of the cell periphery. Although many of the protein parts that make up these adhesion sites were known, scientists had yet to determine how each part fit together to make the overall machine. This was because the building blocks of these machines were both far too small for traditional light microscopes, and far too diverse for electron microscopes.

One of the main protein parts in these machines are the 'cadherin' proteins. The cadherin of one cell extends outside the cell, and interact with cadherin of another cell. On the inside of the cell, cadherin binds to 'adaptor' proteins, which essentially connect the cadherin to a network of protein filaments known as the cytoskeleton. By forging these robust links, cadherin adhesions not only connect neighboring cells but allow cells to coordinate their movements, maintain tissue integrity, and relay a myriad of signals important for proper tissue functions.

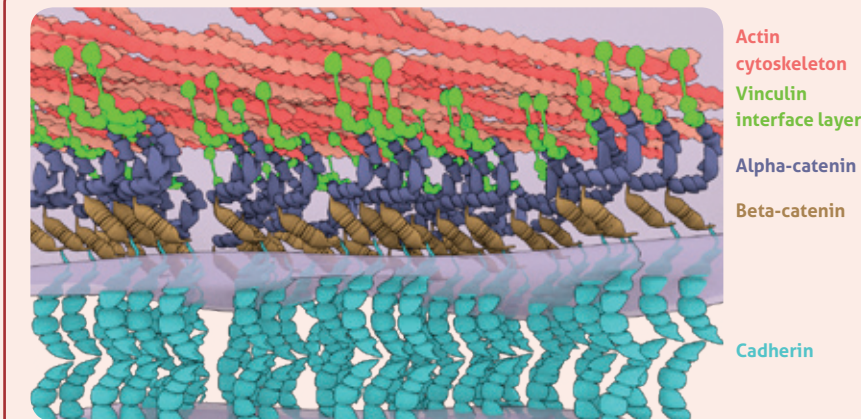


Figure: Compartmentalization of the adherens junction. The core components of the cell-cell adhesion complex are cadherin and the catenin proteins. These are segregated from the actin cytoskeleton, but bridged by an interface layer containing the linker protein vinculin. However, as vinculin is also a mechanosensor, it responds to mechanical and biochemical signals by extending in length, and in doing so, couples the cadherin-catenin complexes to the actin cytoskeleton.

Uncovering the multi-layered organization of cadherin-based cell adhesions

With super-resolution microscopy at their disposal, an international research team led by Asst. Prof. Pakorn (Tony) Kanchanawong from the Mechanobiology Institute and NUS Biomedical Engineering, and Dr. Cristina Bertocchi, Senior Research Fellow at MBI, have revealed, for the first time, how the cadherin-based cell-cell contacts are organized. At the heart of the study is a 'map' of how the parts are pieced together into a sophisticated nanoscale cell-cell adhesion machine. This study is published in Nature Cell Biology.

Here, the researchers 'mapped' the position and orientation of the protein building blocks of cadherin adhesions. They noted a striking degree of compartmentalization in the organization of the protein machinery where components were arranged into multiple layers. Strikingly, the cadherin and the cytoskeleton compartments appeared to be separated by an 'interface layer', which contains vinculin, a stretchable protein which has long been implicated in the cell's ability to sense mechanical force. In this case, Dr. Bertocchi observed

that vinculin could undergo a dramatic shape-shifting transformation, whereby it would switch from a compact shape to a highly elongated form. This elongated form was sufficient to stretch over a distance of 30 nm or more, which was the same distance that cadherin was separated from the cytoskeleton. In a nutshell, vinculin could serve as a bridge to link between the cadherin and actin layers.

Further investigation of this structure highlighted that the shape of vinculin (stretched or compact) was determined by both mechanical tension and biochemical signal inputs. Therefore, the ability of vinculin to selectively engage with a highly dynamic actin cytoskeleton highlights vinculin's role in fine-tuning the mechanical properties of cell-cell contacts in response to varying inputs from the extracellular environment.

The ability to observe, under a microscope, molecular machines such as the cadherin based cell-cell adhesion highlights the power of super resolution microscopy. In this case, the protein parts that make up the cell-cell adhesion have been mapped out, allowing researchers to better understand how cell-cell contacts are formed, maintained, regulated and reinforced to perform vital biological functions.

ABOUT THE RESEARCHER: CRISTINA BERTOCCHI



Senior Research Fellow in the lab of Asst. Prof. Pakorn Tony Kanchanawong at the Mechanobiology Institute. Her research focuses on the role of physical forces in regulating biological systems, and currently she is studying the structure and dynamics of cell-cell adhesions.

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REFERENCE:

Bertocchi C, et al. Nanoscale architecture of cadherin-based cell adhesions. Nature Cell biology (2016) doi:10.1038/ncb3456

Mechanobiology is where mechanics meets biology to understand how physical forces direct biological behaviors. Towards this goal, the Mechanobiology Institute (MBI) was set up 10 years ago in the little red dot of Singapore, and quickly rose to lead the research efforts in this field. At MBI, the research focus is to understand how physical parameters such as force and geometry are able to regulate behaviors across all length scales in the living world - in a single protein, in a bacterium, in a single cell, and in shaping tissues within whole organisms.



The Mechanobiology Institute is a National Research Foundation Research Centre of Excellence located in the T-Lab building at the National University of Singapore.

Forces shaping biology

For over a century scientists have appreciated that forces can shape and repair musculature, bones, and are critical for the development of an embryo (100 years of On Growth and Form, p30-33). Since then, comprehensive understanding of these processes was waiting for the necessary technology. Over the last few decades, science has made great advances in measuring and manipulating forces within biological systems. For example, optical and magnetic tweezers, atomic force microscopy, cell stretching systems, micro and nano fabrication, and laser ablation, combined with high speed and high resolution microscopy has allowed researchers to explore the field of mechanobiology.

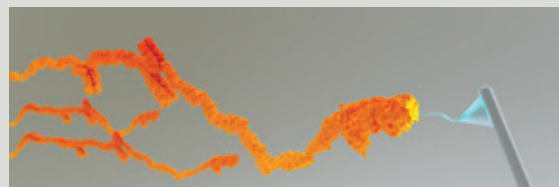
The last century was dominated by genetic and biochemical analyses of biological processes, which led to the discovery of many regulatory signaling pathways. These signaling pathways are composed of proteins that interact with each other in a precisely defined spatio-temporal manner to bring about the desired outcome.

How do these proteins work? One view to tackle this problem is to think of these proteins as a set of complex machines. This concept of molecular machinery is illustrated in the highly dynamic and

complex process of mechanotransduction, which provides a way for every cell in the body to sense and respond to the physical environment. At the core of mechanotransduction is the cytoskeleton, comprised of ancient proteins present in life since prokaryotes (actin, microtubules, and intermediate filaments) that provide structural integrity to the cells. They are polymers which form several different types of networks by binding to different crosslinkers and polymerizing factors. Importantly, cytoskeletal elements are directly coupled to the extracellular matrix, via transmembrane receptors such as integrins. Hence they can sense the physical parameters of the extracellular environment by *mechanosensing*. A family of proteins that can stretch or become enzymatically active upon application of force, the mechanoresponders, help the cells to process this mechanical information by *mechanotransduction*. Hence a physical signal is translated into a biochemical signal, which is then transported to the nucleus to bring about the appropriate gene regulation, a *mechanoresponse*. Misregulation of mechanotransduction can lead to devastating diseases such as cancer, fibrosis, congenital defects, and many others.

An interdisciplinary science

Technological advances help us to visualize and quantify processes occurring at the micro and nano-scale of a cell or protein. A key to this field is also the ability to look at single cells in artificially generated environments that mimic one or more aspects of the natural environment thereby reducing the number of variables - such as stiffness, geometry, flow, which enables us to dissect the real role of the variable under investigation.



Artistic illustration of an optical tweezer being used to stretch a protein to probe its unfolding and refolding kinetics.

Hence, advances in mechanobiology rely on highly interdisciplinary research teams, where theoretical physicists, statisticians computer scientists, engineers, material scientists, and biologists work together to make possible the mammoth task of visualization, and analysis of these processes. Theoretical modeling provides possible frameworks for the workings of these systems. Engineers and material scientists help build the

various devices for micro manipulation and measurement of forces at the desired length scale, and are also responsible for advances in microscopy needed for detailed imaging. Statisticians and computer scientists design effective analytical methods that can interrogate large data sets to extract relevant information. Biologists provide genetic and biochemical understanding, and the 'big picture' view of understanding the biological system. Therefore, an interdisciplinary approach is the key to the success of this venture.

Building this interdisciplinary approach has been, and remains a core principle of the Mechanobiology Institute. As well as hiring and nurturing experts from different disciplines, MBI utilizes the open lab concept, where there are no walls between laboratories, and no designated allocation of space for lab research. This environment facilitates conversations between people from various disciplines, enabling a free and open exchange of ideas that allows for new perspectives and strategies to solve the problems at hand. This is further complemented by core services that run the laboratories, microfabrication, and microscopy facilities that not only ensure efficient and smooth working of such a large institute but also provide important innovations and technological advances – a must for the progress of understanding in this field.



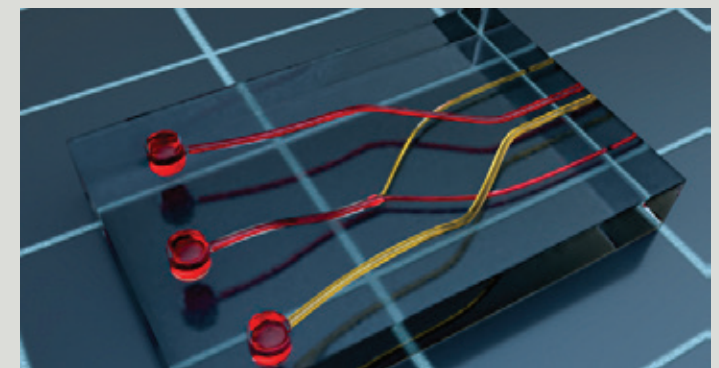
Cover image of Developmental Cell, December 7, 2015. Aboriginal inspired art of nascent adhesions formed on substrates with different rigidities. Illustration by Rishita Changege.

rigid bone, where each of them have connective tissue of a particular stiffness which the cells have to respond to. We have shown that cells lay down universal 100nm adhesion structures on all substrates, irrespective of stiffness, and these form the modular basis of cell adhesions to the surrounding matrix. This discovery would have been impossible without nanoscopy to image these adhesions, or materials science to fabricate different substrates.

Mechanically inspired medicine

The field of mechanobiology has the huge potential for the

advent of modern medicine. Mechanical perturbation offers a new avenue for treatment instead of, or in conjunction with chemical drugs. Importantly, this perturbation can be local, thereby avoiding the systemic side effects of drug treatments. One example that is currently in an advanced stage of discovery is that of fabricated bandages for wound healing. It is now appreciated that regeneration requires an appropriate niche, and this includes physical as well as biological characteristics.



Graphical representation of a microfluidic chip. Microfluidics allows fast, highly sensitive, and cost effective isolation of single cells for disease detection and diagnosis.

Currently, grafts of matrices engineered for desired characteristics are being tested for use as patches to kickstart the regeneration of various tissues, including the spinal cord & retina. For many diseases, early detection can mean that half the battle is already won due to a likelihood of a better prognosis. Several disease states are characterized by loss of the ability to sense the physical environment and these now have the potential to be detected at the early onset of disease. Physical biomarkers therefore can serve as novel markers for early diagnostic tests. Mechanobiology also has the potential to inspire many innovative engineering approaches through bioinspired designs. For example, microfabrication can lead to novel chip designs that can rapidly and cheaply diagnose several disease conditions, nanoneedles similar to those found of body of insects have an intrinsic antimicrobial activity that can be used in conjunction with other disinfectants, and many more which are yet to be discovered.

As a truly interdisciplinary science, mechanobiology promises to bring about new advances in our understanding of the living world, leading the way for research in the 21st century.

ABOUT THE RESEARCHER: RISHITA CHANGEDE

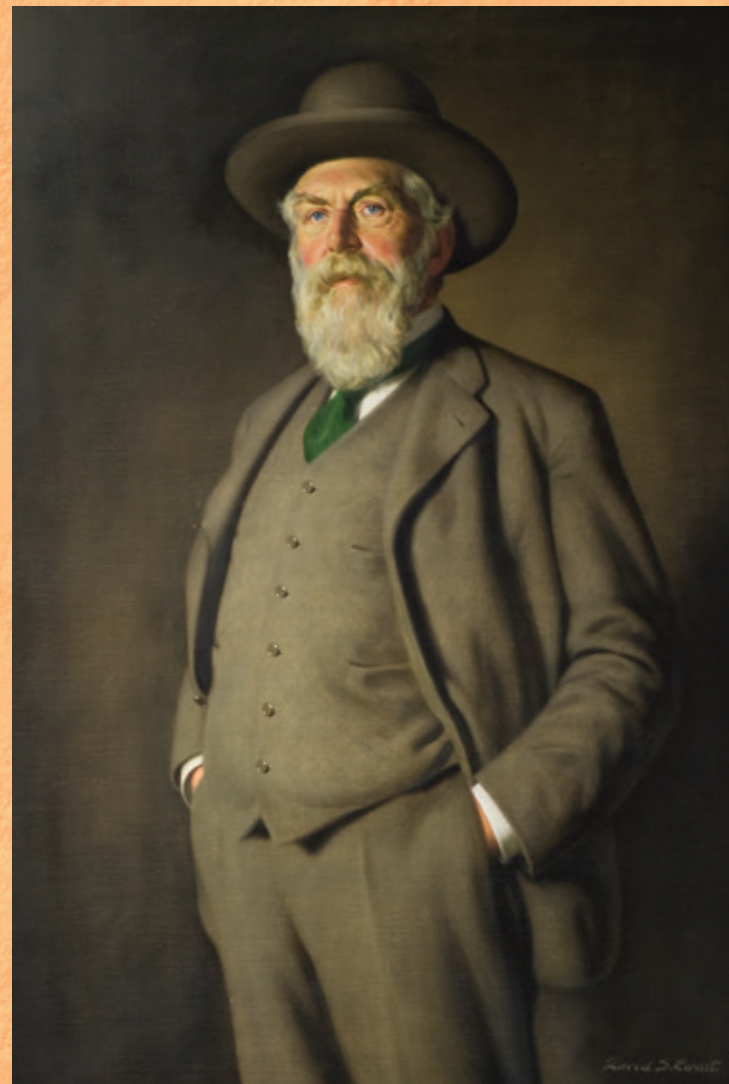


Senior Research Fellow in the lab of Prof. Michael Sheetz at the Mechanobiology Institute. Her primary interest is to understand the design principles that allow the cells and organisms to respond appropriately to their diverse physical (geometry and forces) and chemical (biochemical ligands) environment.

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1 ON GROWTH AND FORM

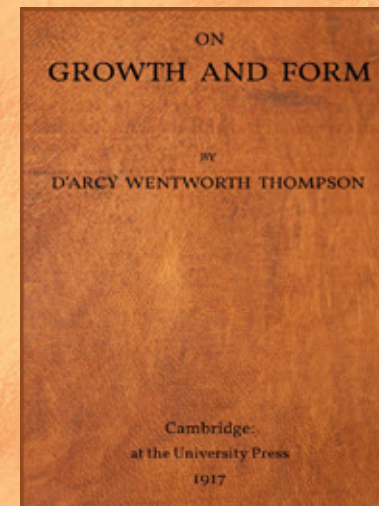
Written by Sruthi Jagannathan. Illustrations by Diego Pitta de Araujo.



Painting of Professor Sir D'Arcy Wentworth Thompson (1860–1948) (after a 1938 original) by David Shanks Ewart (1901–1965). Image courtesy of University of Dundee Museum Services.

"...the fertile field of discovery lies for most part on those borderlands where one science meets another..." – Sir D'Arcy Wentworth Thompson

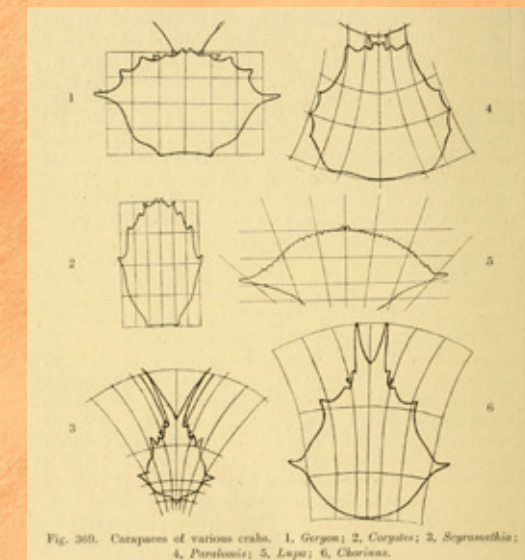
It has been a century since Sir D'Arcy Thompson's magnum opus 'On Growth and Form' was published. Regarded as one of the greatest work of literature in the history of science, 'On Growth and Form' has, since its publication, deeply influenced discoveries and inventions in fields as diverse as biology, arts, anthropology, engineering, computation, and nanochemistry. The ideas presented by Thompson continues to remain a cornerstone of modern scientific theories, inspiring scientists of this era to adopt an integrated, quantitative, and multidisciplinary approach while addressing fundamental questions in biology.



Sir D'Arcy Thompson was a Scottish biologist, mathematician, and classicist, and was a pioneer of mathematical biology. During his career as a Professor of Natural History that spanned over 6 decades, Thompson authored close to 300 books and articles. In his epic work 'On Growth and Form', published in 1917, Thompson drew on his immense and broad knowledge in biology, mathematics, and classics, and framed his masterpiece around one central theme – the shapes or forms of living organisms during development are a consequence of physical principles and mechanical forces.

By using simple, relatable analogies, Thompson drew the underlying connections between physical systems and a broad range of biological phenomena such as the

magnitude, direction and rate of growth, the form and structure of cells and tissues, the formation and functions of diverse forms of spirals, horns, shells, teeth and bone, and the symmetrical arrangement of plant parts around a central axis (phyllotaxis), and so on. However, of all the theories and scientific explanations that he put forth in his book, Thompson came to be most well-known and cited for his theory of transformations. In this groundbreaking theory that inspired scientists, artists, and architects alike, Thompson proposed that differences in the forms or shapes of related organisms (homologous species) can be explained geometrically. The succinct mathematical explanations that he provided in his theory of transformations and in his descriptions of the shapes of various parts of an organism set the stage for many subsequent studies, including the field of evolution-development (evo-devo).



A very significant theme of Thompson's book is his strong emphasis on the importance of mechanics in biological systems as he clearly described the role of physical principles such as force balance and energy minimization in driving biological processes. For instance, using the arrangement of soap bubbles as a simple analogy, Thompson explained the role of surface tension in the packing of cells in the body into hexagonal structures that is

essential for minimizing area and achieving force balance within the biological system. He further used the soap bubble analogy to predict the types of patterns that cells could arrange into in the very early stages of embryonic life, under the influence of physical factors.

He also used analogies to explain the influence of mechanics on the shapes of various organisms. This was exemplified in his descriptions of the shape of a jellyfish, which he related with the forms of drops of liquid falling into a viscous fluid and that of the internal skeleton of birds that he associated with engineering structures (trusses) that provide support to an architecture in the presence of external loads.

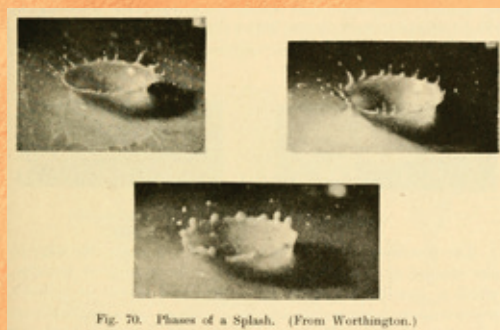


Fig. 70. Phases of a Splash. (From Worthington.)

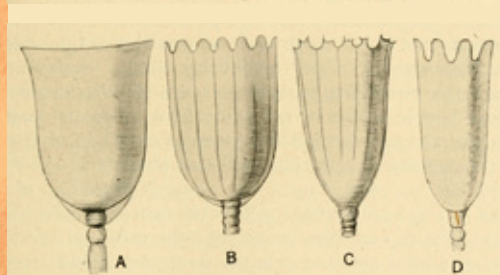


Fig. 72. Calyces of Campanularian zoophytes. (A) *C. integra*; (B) *C. groenlandica*; (C) *C. bispinosa*; (D) *C. varidens*.

D'arcy Thompson described how surface tension is responsible for the similarity between the notches formed at the edge of a splash (top) and the indentations observed in the cup-like shape of a family of jellyfish (bottom).

Such analogies have been useful in understanding the basic mechanisms at play in a number of biological systems; however, since biological processes are much more complex and dynamic than physical systems, there is a growing need to use advanced quantitative techniques and interdisciplinary approaches to unravel the finer molecular details that drive these processes under physiological conditions.

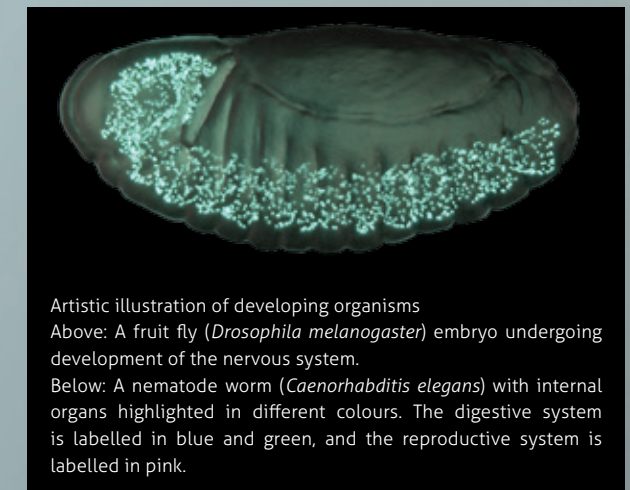
A century later, from theories to mechanobiology

The modern, interdisciplinary field of Mechanobiology, which is a study of the mechanics of biological systems, embodies the fundamental philosophies laid out by Thompson. Needless to say, research in this field has been greatly influenced by many of Thompson's ideas, and as pioneers in this emerging, multidisciplinary field, the central theme of research at the Mechanobiology Institute emulates many ideas and proposals put forth by Thompson.

Researchers at the MBI are, for example, applying advanced techniques such as micro-engineered substrates to manipulate one or more physical parameters of the tissue microenvironment. Super-resolution microscopy techniques and other quantitative tools are then used to visualize the force-induced conformational changes in proteins, as well as their dynamic assembly into distinct subcellular structures. Such approaches can determine the role of these structures in sensing and relaying physical or mechanical signals across the cell. Studies are also ongoing to unravel the complex interplay of biochemical signaling pathways that are activated in response to physical signals from the tissue surroundings, and to identify the major proteins involved in these pathways. By focusing on the molecular mechanisms responsible for the communication of physical signals during fundamental biological functions, these recent studies add a level of enquiry that was beyond the scope of available scientific tools when Thompson put forth his theories highlighting the significance of mechanics on biological systems.

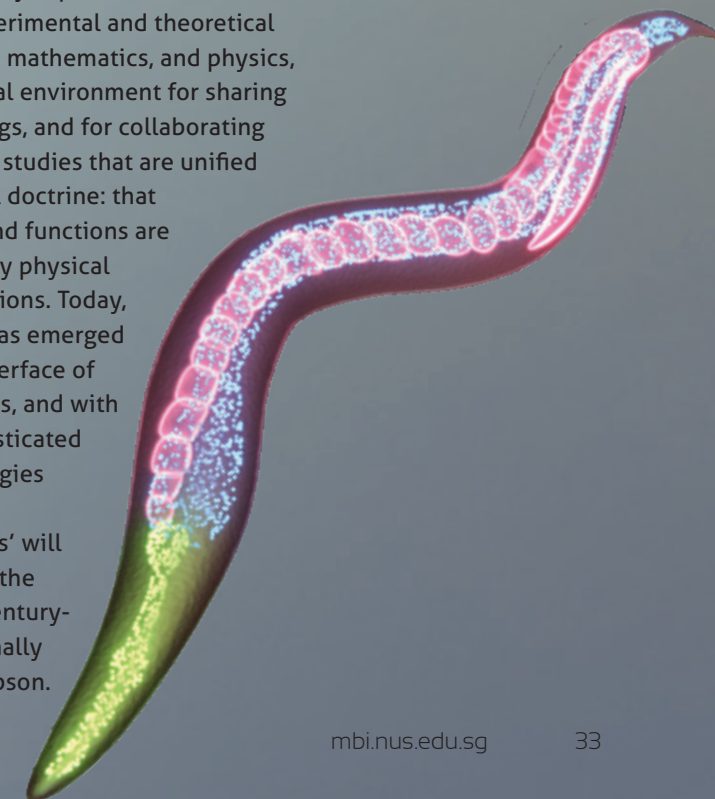
100 years on and our ability to examine the mechanical forces at play during biological processes continues to be enhanced, not only by improved imaging techniques that allow for very high spatial resolutions (to the level of single proteins), but also by techniques that allow researchers to examine processes over increasingly long time scales. At MBI a novel light-sheet microscope has been developed in collaboration with researchers in France, that enables high quality and long-time imaging of cells. MBI also has a custom-

built multi-view light-sheet microscope that enables high spatio-temporal resolution imaging of developing organisms throughout embryogenesis. With these latest imaging techniques, MBI researchers are now able to, for example, examine the cellular and tissue-scale events that lead to the formation of complex organ shape during development. In collaboration with the theoretical physicists working at MBI, the massive amounts of experimental data generated are analyzed mathematically, and built into robust models - similar in theme to D'Arcy Thompson's scientific principles - but now based on well-defined molecular and cellular events, thus providing more accurate reconstruction of the various stages in development.



Artistic illustration of developing organisms
Above: A fruit fly (*Drosophila melanogaster*) embryo undergoing development of the nervous system.
Below: A nematode worm (*Caenorhabditis elegans*) with internal organs highlighted in different colours. The digestive system is labelled in blue and green, and the reproductive system is labelled in pink.

The multidisciplinary expertise of MBI researchers in experimental and theoretical biology, chemistry, mathematics, and physics, has created an ideal environment for sharing of ideas and findings, and for collaborating on a wide range of studies that are unified around one central doctrine: that biological forms and functions are greatly impacted by physical forces and interactions. Today, mechanobiology has emerged as a field at the interface of biology and physics, and with increasingly sophisticated tools and technologies at their disposal, 'mechanobiologists' will continue to reveal the significance of a century-old ideology originally put forth by Thompson.



CONTROLLING MEMBRANE WAVES

Written by Andrew Wong. Illustration by Diego Pitta de Araujo.

Many biological functions rely on the establishment and maintenance of biochemical rhythms. Some examples include the cell cycle, which enables regular turnover of new cell growth. Many organisms, including humans, follow a 24 hour cycle of activity and rest based on circadian rhythms. On the cellular level, rhythmic oscillations in the cell membrane are linked with changes in cell shape, locomotion, and development. Based on mathematical principles, oscillations are generated from pulses of activity from an activator coupled with that of the inhibitor, and the inhibitor is essential for determining the spatiotemporal pattern of oscillation. However, in biology, much of the research to date has been on identifying activators, rather than investigating inhibitory mechanisms.

ABOUT THE RESEARCHER: MIN WU



Principal Investigator at the Mechanobiology Institute, Centre of Bioimaging Sciences, and Assistant Professor at the Department of Biological Sciences, NUS. The Wu lab investigates how biological membranes are shaped and how membrane geometry affects cell function.

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REFERENCE:

Xiong et al., Frequency and amplitude control of cortical oscillations by phosphoinositide waves. Nat Chem Biol. 2016 Mar;12(3):159-66. doi: 10.1038/nchembio.2000.

Rhythmic oscillations in cell biology

In cellular systems, the pattern of oscillation observed frequently is that of periodic travelling waves. This pattern is generated from a moderate spread of an activator, and short-range activity of an inhibitor. Periodic travelling waves independently develop in rat basophilic leukemia cells, a type of white blood cell. In order to investigate how oscillations are regulated at the molecular level, a team of MBI scientists led by Asst. Prof. Min Wu from the Mechanobiology Institute, National University of Singapore, used fluorescently labelled protein to visualise periodic travelling waves in the membrane of these leukemia cells.

Microscopic analysis of these waves, coupled with biochemical assays and optogenetic investigation allowed the scientists to identify the activators and inhibitors responsible for wave dynamics. The amplitude, or strength of the wave was driven by pulses of the lipid PtdIns(4,5)P₂, whereas the frequency of the wave was set by another lipid, PtdIns(3,4)P₂. As hypothesized in the mathematical model, these pulses are controlled by local inhibitor activity. The

researchers identified two inhibitors, namely synaptojanin 2 and SHIP1, both of which are enzymes which can break down lipids, providing a clear mechanism for generating pulses of activator lipid.

This study represents a key step towards understanding the molecular mechanism of cortical pattern formation, although it is likely that more activators and inhibitors remain to be discovered. Aside from their importance in regulating fundamental biological processes such as cell growth and migration, the patterns of cortical oscillations also enable the cell to regulate signaling dynamics. For instance, the activity of PI3K p110 δ , the major functional PI3K isoform for the waves, is not proportional to the amount of PtdIns(3,4,5)P₃ on the plasma membrane. Rather, it is encoded by the frequency of PtdIns(3,4,5)P₃ pulses.

Considering that p110 δ has been the most successful clinical PI3K target so far for blood cancer drug development, and that SHIP1 and synaptojanin 2 have a well-established role in leukemia and other cancers, amplitude and frequency-based information processing offers a novel framework for dissecting the complex fine tuning of cellular signaling in health and disease.

DEFECTS IN EPITHELIAL TISSUE ORGANIZATION

A QUESTION OF LIFE OR DEATH

Written by Steven Wolf. Illustrations by Melanie Lee, Diego Pitta de Araujo, and Chun Xi Wong.

HOW THE MISALIGNMENT OF CELLS PREDICTS CELL DEATH AND REMOVAL IN EPITHELIAL TISSUE

An international team of scientists from the Mechanobiology Institute, National University of Singapore have discovered the primary mechanism driving the extrusion of dying cells from epithelial monolayers. This work has been published in Nature.

The removal of cells from a tissue occurs regularly. Not only are damaged or dying cells removed, but the process of cell extrusion can prevent regions from becoming overcrowded. This is particularly important not only during developmental processes when tissues and organs are being formed, but also in diseases such as cancer, when tumors grow uncontrollably. Despite the importance of cell extrusion in development and aging, as well as the pathological importance in cancer progression, the cues that flag a cell for removal were poorly understood.

Now, by studying single-layers of epithelial cells grown in the lab, scientists from the Mechanobiology Institute, National University of Singapore, and Institut Jacques Monod, CNRS and University Paris Diderot (France) in collaboration with researchers from Oxford University (UK) and Institut Curie (France) have found that the major factor driving cell death and removal relies on the physical arrangement of cells in the surrounding cell layer. In particular, the appearance of defects in the cellular patterns of epithelial layers promotes cell death and elimination from the tissues.

There are several examples in nature where a molecule or cell type aligns in a defined manner. Bacteria colonies, fat molecules, and even internal components of the cell, are just a few examples. Another well-known example corresponds to liquid crystals, a state of matter between a solid and a liquid, which can consist of rod-shaped

molecules. Under certain conditions, these molecules can align along a preferential orientation when altered by electric, magnetic fields or temperature.

Liquid crystals in epithelial tissue

This phenomenon is particularly well-known since it is exploited in technologies such as liquid crystal displays. In this case the optical properties of liquid crystals are determined by their alignment. Shifts in their alignment determine what we see on the display. As in any crystal, a perfect arrangement does not exist in liquid crystals and defects emerge in their arrangement that strongly modify their physical properties. The starting point of this study was to show how the behaviour of the cell sheet is analogous to liquid crystals.

Here, PhD candidate Thuan Beng Saw, together with Prof. Chwee Teck Lim of the MBI, and Prof. Benoit Ladoux of the Institut Jacques Monod (IJM, CNRS) and MBI, in collaboration with Dr. Doostmohammadi and Prof. Yeomans (Oxford), Prof. Marcq (Curie Institute, France) and Asst. Prof. Toyama (MBI), found that like the liquid crystals in a phone or laptop monitor, epithelial cells were arranged parallel to each other with their 'long' sides all facing the same direction.

Following this analogy, they also observed the emergence of 'topological defects', which caused the cells to realign so that they resembled a comet. In this case, cells at the head of the comet pattern had shifted so that they now aligned perpendicular to the cells that made up the tail. Some cells turned up to 90 degrees. In a liquid crystal display, such realignments of the molecules merely alter the optical properties of the material. However, in

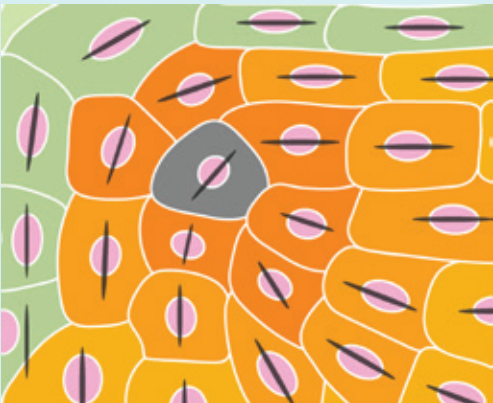
an epithelial sheet, such changes in the pattern can mean life or death for the cells involved. Remarkably, it was after this cell realignment that cells near the head of the 'comet pattern' died and were removed from the surrounding tissue.

To further investigate the relationship between cell death and topological defects, they examined the forces being generated around these particular areas of cell misalignment. They found that compressive force concentrated at the head of the comet pattern. This force generated over an hour prior to cell extrusion, and was sufficient to trigger cell death at topological defects. As cells are connected to each other by protein cables and adhesions structures, any movement of a cell causes tension to be propagated to its neighbours. The misalignment of cells causes significant bending of cells and this leads to high compressive stresses in these regions. These stresses are sufficient to trigger apoptosis and cell extrusion of a nearby cell.

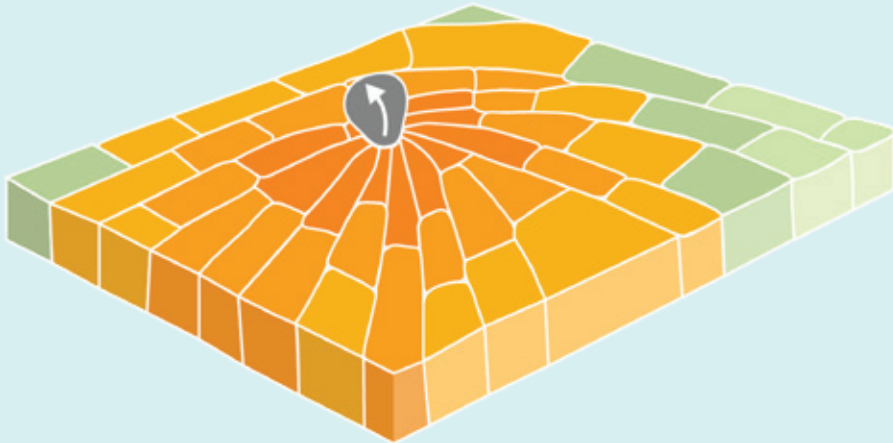
Tissue engineering and regenerative medicine requires scientists to carefully control cell growth and tissue development in a lab. The findings presented in this work are of fundamental importance towards achieving this control. Indeed, the researchers successfully controlled how cells aligned by introducing shapes in areas where the cells grew that mimicked the topological defects associated with cell death and extrusion. This allowed them to pinpoint where in the cell sheet extrusion would occur. These discoveries provide a significant step forward in our understanding of how the physical microenvironment plays a role in tissue development, and provides new approaches with which researchers can control, analyze and study cell growth and death.



A. Monolayer epithelial tissue



B. Emergence of a topological defect and comet realignment



C. Cell extrusion from the tissue

Figure: Schematics showing the arrangement of cells in normal epithelial tissue (a) in a parallel alignment. Following the introduction of a topological defect (b), the cells realign in the shape of a comet. This causes a concentration of compressive force (orange gradient), and leads to cell death and extrusion in the epithelial layer (c).

ABOUT THE RESEARCHERS: CHWEE TECK LIM



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BENOIT LADOUX



Co-Principal Investigator at the Mechanobiology Institute, and Professor at the Institut Jacques Monod, Université Paris Diderot & CNRS, France. The Ladoux lab studies the cooperation between adhesion, biomechanical, and biochemical signaling for the adaptation of living organisms to changes in their environment.

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YUSUKE TOYAMA



Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biological Sciences, NUS. The Toyama lab uses interdisciplinary techniques, such as imaging, gene manipulation, and biophysical modeling to extract mechanical signatures from cell and tissue dynamics.

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REFERENCE:

Saw et al., Topological defects in epithelia govern cell death and extrusion, Nature 544, 212–216 [12 April 2017] doi:10.1038/nature21718).

TO DEEP TO DIVIDE

HOW CELLS SENSE DEPTH AND HALT CELL PROLIFERATION

Written by Lakshmi Ramachandran and Steven Wolf. Illustration by Diego Pitta de Araujo.

A collaborative, interdisciplinary study by the research groups of Assoc. Prof. Boon Chuan Low and Prof. Chwee Teck Lim at MBI, National University of Singapore, has revealed that both normal and cancer cells are able to sense the depth of pores in bones, and in response, trigger distinct mechanosignaling pathways that can halt cell growth and division. This work is published in *ACS Nano*.

Molecular response to depth sensing differs in cancer cells

Cancer is a disease that is characterized by the uncontrolled growth of cells. In some cases, cancer cells can spread from the primary tumour location, for instance, the breast, to secondary locations such as bone, brain, liver or lung. This occurs through a process known as 'metastasis' and is often attributed to a poorer prognosis for patients with advanced

stage cancers. When cancer cells become metastatic, they not only gain the ability to move to regions of the body that are physically and biochemically distinct from the original tumour, but can adapt to these new conditions. In some cases, cells can move between environments of completely opposing properties. For example, cells that normally grow in the soft breast tissue may establish a secondary tumour within the much harder environment of the bones. In these cases, the physical properties of the new regions dictate the behaviour of the cancer cells, and promote or halt their growth and division, or cause them to move on to other sites.

Now, in research published in the journal *ACS Nano*, MBI graduate student Parthiv Kant Chaudhuri, has shed light on how topographical features found on secondary tumour sites such as bone, influence mechanisms within the cellular machinery that drives the growth and division of cells. Specifically, the

researchers analysed the mechanical effect of bone pore dimensions on the dynamics of the cytoskeleton during the proliferation of normal vs cancerous breast cells.

In earlier work from the MBI, the same researchers showed how features that are commonly encountered by cells, such as furrows, ridges or pores, can affect the proliferation of cells. Here, a phenomenon called Mechanically-Induced Dormancy (MID), was described, where normal breast cells, but not breast cancer cells, stopped dividing on an artificially created topography that mimicked the breast cancer microenvironment. This discovery opened up the possibility of identifying novel anti-cancer strategies that target cellular mechanisms altered by mechanical cues during cancer progression.

To further their research on MID, and understand how the physical properties

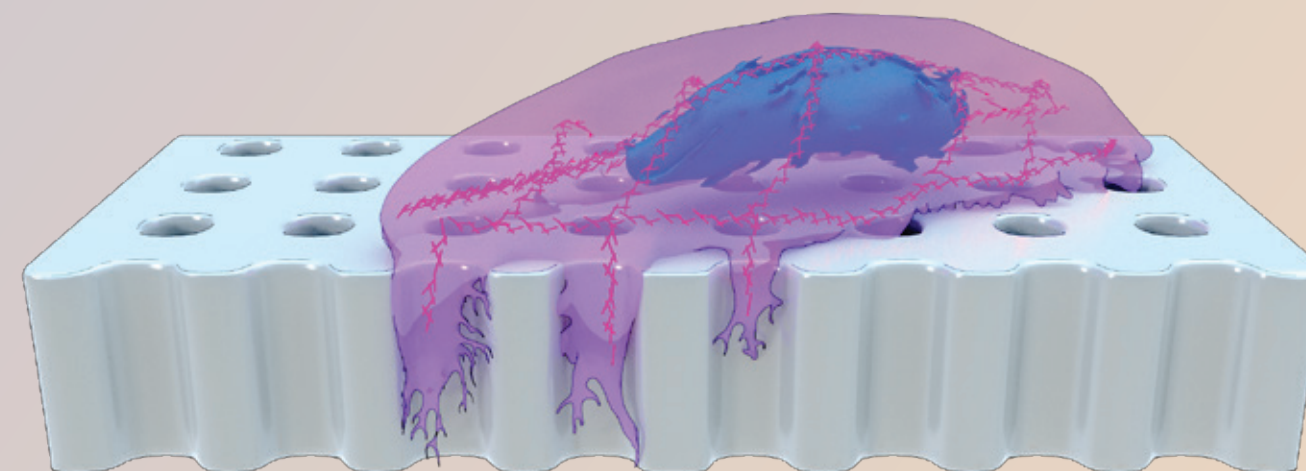


Figure: Artist's impression of a malignant cell extending finger-like protrusions (invadopodia) into micropits that mimic bone surface. These protrusions sense pore depth, and at a specific depth, prevent cell proliferation in cancer cells via decreased cytoskeletal (red filaments) contractility.

of bone influence breast cancer cell growth, the researchers fabricated pits of varying but defined width and depth, to mimic the porous matrix of the bone. Normal and malignant breast cells were grown on these micropits and were analysed by confocal microscopy. The team noted how the cells sent out finger-like protrusions into the micropits. They characterized these protrusions as 'invadopodia' which are cellular structures rich in a cytoskeletal protein called actin, that are put forth by cancer cells to enable sensing and degradation of the cancer cell's microenvironment.

The researchers noted that at a specific depth, the sensing carried out by invadopodia initiated a halt in cell proliferation. This was observed in both cancer and non-cancer cells and was similar to the previously described phenomenon of mechanically induced dormancy (MID).

Although the response of both cell types was to halt their growth, it remained possible that the molecular mechanisms driving MID differed between the two cell types. Such differences would be consistent with what is known about

cancer cell behaviour; that they often display erratic or uncontrolled protein activity in response to otherwise normal stimuli.

Indeed, when the researchers examined the molecular mechanism at play, they found that the cancer cells initiated MID by reducing the contractility of the cytoskeleton, which was in contrast to the normal cells, where contractility was increased. Furthermore, they found that the inhibition of contractility using chemical inhibitors further reduced the proliferation of cancer cells.

The findings from this study highlight the importance of understanding how cancer cells respond, at the sub-cellular level, when they relocate and encounter new and foreign environments. Here, the molecular responses to depth sensing differed from normal cells even though the ultimate outcome for both cells types was a halt to their proliferation. Such anomalies in sub-cellular behavior may provide novel targets for new anti-cancer therapies, and be particularly useful in preventing cancer cells from adapting and growing in new areas of the body.

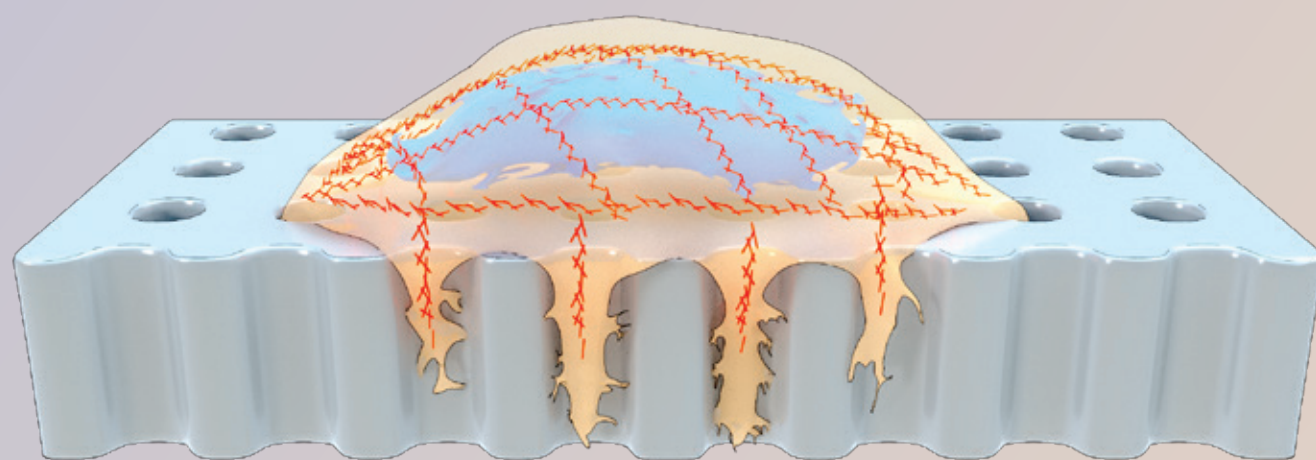


Figure: Artist's impression of a normal cell extending finger-like protrusions (invadopodia) into micropits that mimic bone surface. These protrusions sense pore depth, and at a specific depth, prevent cell proliferation in normal cells via increased cytoskeletal (red filaments) contractility.

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A MOVING STORY OF FHL2 AND FORCES

Written by Sruthi Jagannathan. Illustration by Diego Pitta de Araujo.

Researchers from MBI, National University of Singapore have revealed the molecular events leading to the regulation of cell growth and proliferation in response to stiffness of the extracellular matrix that surrounds them. This study is published in the Proceedings of the National Academy of Sciences, USA.

How does matrix rigidity influence cell proliferation?

It is becoming increasingly clear that cells react to the mechanical properties of their immediate micro-environment, which

is essentially a molecule-based scaffold called the extracellular matrix (ECM). A greater emphasis is now being placed on how ECM properties, such as its stiffness, shape, and curvature, can influence fundamental cellular behaviours; in particular the ability of a cell to grow, proliferate, adopt specialized functions and move around.

In many cases, researchers are able to see a story emerge, where a cell becomes aware of its surroundings, and responds in a particular way. In many of these stories however, we can only see the start and end of a highly complex series of events; the intermediate molecular events that connect the mechanical signals to the cellular response remain largely unclear.

Unraveling the molecular story that unfolds when a cell responds to one ECM property – its stiffness – was the focus of a study led by MBI Director Prof. Michael Sheetz and Postdoctoral Fellow Naotaka Nakazawa.

In this case they were particularly interested in how ECM stiffness influences the synthesis of proteins related to cellular proliferation.

As their story developed, a main character emerged; a protein called FHL2, and a specific molecular event; its relocation from sites near the cell membrane to inside the nucleus. At the membrane, FHL2 helps the cell attach to the ECM and receive mechanical signals, however once inside the nucleus it binds to specialized molecular machinery on the DNA, and drives the decoding of genes.

By using advanced laboratory techniques such as atomic force microscopy to measure the stiffness of the ECM, and super-resolution imaging to visualize the sub-cellular location of individual proteins, the researchers revealed that the movement of FHL2 into the nucleus would only occur if the cell was unable to apply enough force onto the ECM. In other words, FHL2 moved into the nucleus when cells were surrounded by a soft ECM, or when cells were unable to generate enough force to move against the matrix.

At this point, it became clear that the transport of FHL2 to the nucleus could only occur if the protein was chemically modified first. This was carried out by another protein called Focal Adhesion Kinase (FAK) in response to changes in the mechanical forces surrounding the cell. Once inside the nucleus, FHL2 was

free to carry out its role in helping decode the DNA, which in this case was to turn on the synthesis of a specific protein called p21, an inhibitor of cell growth and proliferation.

Erratic cell growth in soft environments is associated with cancer cell proliferation and tumour growth. For a healthy cell to detect a soft environment, and ensure erratic cell growth does not occur, it requires proteins like FHL2 to mediate the production of proteins like p21.

While this story ends with the protective action of FHL2 against cancers, in other cases its prominent role in cell signaling becomes a double-edged sword. For example, in breast and ovarian cancers, FHL2 is known to counteract mechanical signals and promote cancer cell growth and metastasis.

It is therefore imperative that research continues to dissect the molecular pathways involving this protein. With the story of how FHL2 suppresses the growth of healthy cells in soft environments now told, it is hoped that researchers can probe why it behaves differently in cancer cells and whether targeting these molecular events can prove beneficial to cancer patients.

ABOUT THE RESEARCHER: MICHAEL SHEETZ



Director of the Mechanobiology Institute, Distinguished Professor of the Department of Biological Sciences, NUS, and Emeritus Professor of the Department of Biological Sciences at Columbia University, USA. The Sheetz lab focuses on understanding the molecular mechanisms of mechanotransduction and their involvement in a variety of phenomena from cancer metastasis to brain function.

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BREAKING CELL SYMMETRY

A FORCE DRIVEN MECHANISM FOR ESTABLISHING CELL POLARITY

Written by Andrew Wong. Illustration by Melanie Lee.



A team of researchers from the Mechanobiology Institute, National University of Singapore, along with colleagues from Temasek Life-sciences Laboratory and the Institute of Molecular and Cell Biology, A*STAR in Singapore, reveal a novel mechanism for establishing cell polarity that relies on tension force induced clustering of proteins. This study was published in Nature Cell Biology.

Biological cells are typically visualized as round (or spherical) in shape, with a nucleus centred in the middle, and other cellular components scattered throughout. In reality, each cell type exhibits a distinct shape, size and composition. Depictions of symmetrical spheres is in essence an oversimplification that hides the fact that nearly all cells are asymmetric in their composition, and that this asymmetry develops in precise, and well-ordered, steps.

Known as cell polarity, this key characteristic of cells sees the separation of the sub-cellular components into distinct regions of the cell. If cells were symmetrical, processes like the division and movement of cells would not occur correctly, and tissues and organs would be deformed and non-functional. Despite being integral to organism development, scientists have yet to fully define the processes by which cells become polarized.

One way to visualise the asymmetrical nature of cellular composition is to think about the components of a car, and how they are arranged. Some parts of the car have to be located in a balanced layout, for instance the wheels. Other components need to be arranged in a specific orientation to work properly, i.e. the driver's seat has to be located in front of the rear passenger seats. Finally, components such as the engine can be located in the front or back of the car, and importantly this organization imparts different properties to the car's handling. In the same way, the arrangement of cellular components can have drastic effects on cell function.

Many of the existing studies on cell polarity have been carried out in the nematode worm *C. elegans*. At the one-cell stage, the embryo divides along a front/back axis to generate two differently sized daughter cells, with a larger cell at the front and a smaller cell at the back. This front/back axis is established by the movement and segregation of a group of proteins known as PAR (partition defective) proteins.

These PAR proteins reside in the cell cortex, a dynamic layer of protein filaments that lies just inside the cell membrane. Before polarization, the PAR proteins are distributed throughout the cortex, where they move freely. During polarization, the cortex contracts, and this causes different PAR proteins to separate,

and accumulate at either the front or back of the cell, thereby breaking their previously symmetrical organization and establishing polarity along the front/back axis. However, the mechanism by which contractile activity transports and segregates PAR proteins remains unclear.

Tension flow

A team of researchers led by Asst. Prof. Fumio Motegi, Principal Investigator at MBI and Temasek Life Sciences Laboratory sought to answer this question by observing the movement of fluorescently labelled PAR protein complexes under the microscope in live *C. elegans* embryos as they underwent polarization. Using advanced microscopy techniques, they discovered that certain PAR proteins assembled into clusters at the beginning of polarization, and these clusters grew in size as polarization progressed. Once cortical contraction stopped, the clusters disassembled, with the proteins spreading out as a gradient along the front/back axis.

Despite these findings, the researchers did not observe a direct connection between the contractile fibres and PAR proteins, and this led them to hypothesize that an indirect effect of contraction was responsible for clustering. By disrupting or reinforcing the actomyosin cortex and observing the effect on cluster formation, they discovered that the key force driving PAR clustering was cortical tension, which developed as the cortex contracted.

From this the researchers were able to propose a new model that explained the segregation of PAR proteins. Here, contraction of the actomyosin cortex leads to an increase in cortical tension, causing the PAR proteins to assemble into clusters. As these large clusters move slowly, they become caught up in

the overall cortical flow and segregate at one end of the cell, thereby establishing polarity. These segregated clusters of PAR proteins then act as a scaffold that mediates a local accumulation of other proteins needed for the establishment of front/back axes along the body.

The mechanism discovered in this study is a simple yet elegant example of how cells use internal forces to move and organize their protein components in a precise, well-ordered manner. Importantly, the force-driven mechanism described allows the cell to establish polarity without wasting energy by actively transporting proteins or cellular components against a concentration gradient. It is believed that similar mechanisms are used to break symmetry in other organisms, including humans, and it is hoped that this new knowledge will help scientists understand how and why cell polarity fails to be properly established in diseases such as cystic fibrosis and cancer.

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HER2 CAN HEAR2

HOW THE 'DEAF' RECEPTOR RESPONDS TO MECHANICAL SIGNALS

Written by Steven Wolf and Sruthi Jagannathan. Illustration by Diego Pitta de Araujo.

ABOUT THE RESEARCHER: MICHAEL SHEETZ



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REFERENCE:
Saxena M, et al. EGFR and HER2 activate rigidity sensing only on rigid matrices *Nature Materials*, 1 May 2017. doi:10.1038/nmat4893.

Scientists from the Mechanobiology Institute, National University of Singapore along with colleagues from Columbia University, USA have identified the role of ERBB family receptors, including EGFR and HER2 in integrating mechanical and biochemical signals that are essential for promoting cellular behavior such as its growth and movement. This study is published in *Nature Materials*.

HER2 activates mechanosensing on rigid matrices

In recent studies from the Mechanobiology Institute, National University of Singapore, the mechanisms cells use to measure the physical properties of their surroundings were described for the first time. The

studies, which were conducted by researchers from the laboratory of Prof. Michael Sheetz, revealed subcellular contractile machines that pinch on the material surrounding cells as a means of determining how soft or stiff the environment is. This process is now widely known as mechanosensing, and evidence is mounting to suggest that the information gathered can define cellular growth patterns, initiate cell death programs, and even instruct cells on the need to relocate to other regions in the tissue.

Of course, our bodies, and the tissues in which individual cells reside, are highly dynamic, and physical or mechanical cues, are just one factor that determine cell behavior. Both inside and outside of cells, the environment is teeming with biochemical molecules; from cytokines to hormones, and even small chemical

molecules such as carbon monoxide or hydrogen sulfide. Not surprisingly, the process of mechanosignaling is intertwined with biochemical signaling. Despite having described key machines within the cell that facilitate mechanosensing, the researchers had yet to crack one key question; understanding what was controlling the contractile pinching machines. To answer this question MBI Research Assistant Yang Bo Beverly from Prof Sheetz' lab, turned her attention to exploring the influence of biochemical signalling on the activation of the contractile mechanosensors.

Of particular interest was the role of a protein known as HER2, as its levels are often abnormally increased in cancer cells, and cancer cells often exhibit altered growth patterns, disrupted cell death programmes and increased motility.

The HER2 protein belongs to a larger family of specialized receptor proteins known as the ERBB family, HER2 was known to detect signals from outside the cell, and convey these signals to the nucleus. However, HER2 was also unique from other members of the

ERBB family, as it does not bind to any known signalling molecule and can only pass on biochemical information when in the presence of another ERBB family member. It had become known by scientists as a 'deaf' receptor.

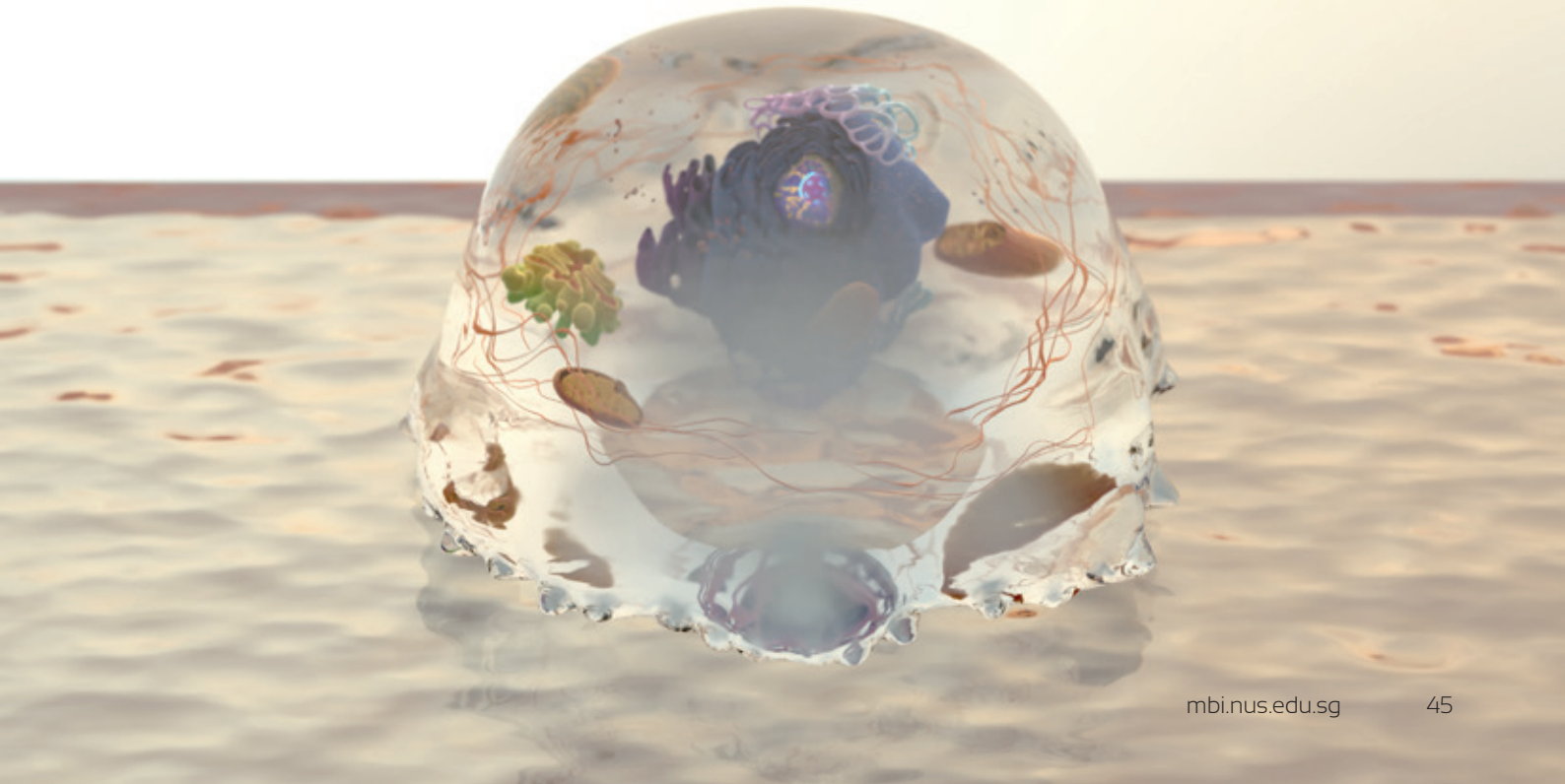
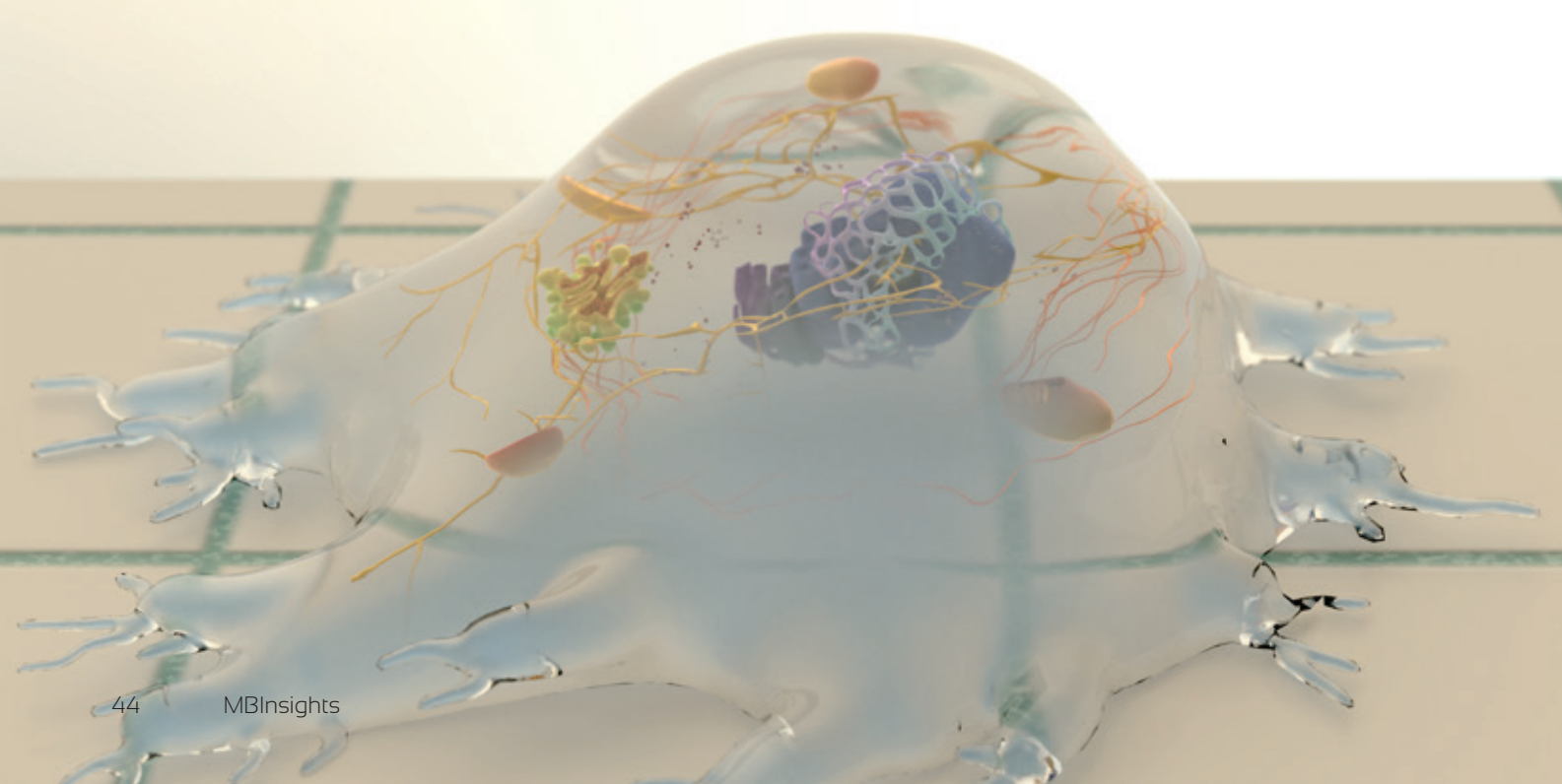
However, as Yang and colleagues revealed, HER2 is indeed responsible for the activation of the pinching machines, even when other ERBB family members were absent. More importantly, activation by HER2 only induced rigidity sensing contractions when the cells were on hard surfaces, and not on softer tissues. This suggested that HER2 was not 'deaf' after all, but rather, it 'hears' a different type of signal. HER2s ability to detect mechanical signals was independent of other ERBB receptors, and allowed the cell to respond by increasing contractions, to subsequently promote cell growth and movement.

It remains possible that the increased levels of HER2 in cancers contribute to the altered response of cancer cells to mechanical signals. However, what is more important, is the complex relationship between mechanical and biochemical signalling, and how

this relationship can be disrupted in cancer. Not only can mechanical signals drive biochemical pathways, but the biochemical pathways can in turn control the cellular mechanosensing machines. Any disruption to these controls can lead to a misinterpretation of the mechanical cell environment, and the erratic cell growth commonly associated with cancer.

Previously, the HER2 signalling pathway was targeted using single-drug therapeutic approaches, however these drugs failed to effectively prevent cancer growth. From the work presented in this study it becomes clear that the impact on the ability of cells to sense and respond to their physical environment must be considered in the development of future cancer drugs that target biochemical receptors.

Figure: HER2 activates mechanosensing on rigid surfaces even in the absence of other ERBB family members such as EGFR. While HER2 enhances actomyosin dependent contractions and cell spreading on hard surfaces (facing page, left), it did not have any effect on cellular contractility and spreading on soft surfaces (this page, right).



SOSPIM-FCS

A NOVEL TOOL FOR IMAGING NUCLEAR PROTEIN DYNAMICS

Written by Sruthi Jagannathan. Illustration by Melanie Lee.

A joint study between scientists at MBI, National University of Singapore and the Interdisciplinary Institute for Neuroscience, France, has resulted in the development of a novel technological approach for observing the complex behavior of proteins inside the nucleus and analyzing their effect on cellular functions. This study was led by MBI Research Fellow Dr. Anand Pratap Singh and IINS Researcher Dr. Remi Galland along with MBI Principal Investigators Asst. Prof. Timothy Saunders and Assoc. Prof. Virgile Viasnoff, and was published in the Biophysical Journal.

Tracking protein movement in time and space

The nucleus is the information processing center of a eukaryotic cell. It contains various sub-nuclear compartments, each of them housing specific DNA regions and proteins. The diverse functions of the nucleus are facilitated by the sharing of protein machineries between nuclear compartments. A vast number of nuclear proteins roam the nucleus, rapidly associating and dissociating with other proteins or DNA segments. This enables the nucleus to process different signal types, such as chemical, mechanical, and electrical signals, and in response, turn specific genes on or off.

Observing the movement of proteins inside the 3D space of the nucleus would reveal valuable information on their specific biological

roles. Despite the advent of high power imaging techniques that allow researchers to visualize the dynamics of fluorescently labeled single proteins in living cells, their use has been limited due to their phototoxic effects on cells. Phototoxicity is induced by prolonged exposure to light, whereupon fluorescent molecules, in their excited states, tend to react with molecular oxygen and form free radicals that can damage subcellular structures.

In order to get past this problem, researchers at the Mechanobiology Institute, Singapore and the Interdisciplinary Institute for Neuroscience, France combined a microscopy method that they had recently developed, known as so-SPIM (single objective selective plane illumination microscopy), with FCS (fluorescence correlation spectroscopy), an analytical technique for measuring protein dynamics.

What is so-SPIM and FCS?

so-SPIM allows the sample (a single cell in this case) to be illuminated along a narrow focal plane and images are captured by an objective lens placed perpendicular to the sample. The sample is then moved through the light beam to expose different parts or sections for visualization at any given time. Since this technique restricts the light beam to a narrow section of the sample, so-SPIM minimizes phototoxicity.

FCS measures the brightness of light emitted by proteins tagged with fluorescent dyes at different points in time. Any variations in brightness over the observation period is linked to the movement of proteins in time and space.

How can these methods overcome phototoxicity?

By combining the two techniques, the researchers were able to measure the brightness of light emitted by fluorescently-labeled proteins at

multiple spatial locations within the nucleus. Any fluctuations in brightness were inferred as changes in protein concentrations within the nuclear area under observation. Using this data, the researchers were able to identify the movement of proteins within the 3D space of the nucleus over a set period of time. Specifically, the proteins moved from regions where they were highly concentrated, to regions of lower concentration. When movement was slow, the researchers could tell that the protein was interacting either with other proteins, or with strands of DNA or RNA. In these cases the protein would be catalyzing gene expression or protein synthesis.

Applying the technique

The researchers used the soSPIM-FCS setup to observe the dynamics of two proteins inside the nucleus, namely RNA Polymerase II and YAP. RNA Polymerase II (Pol II) is a transcription factor—it is responsible for decoding information on DNA into intermediary RNA molecules, which is then translated into proteins. YAP, on the other hand, is a transcriptional coactivator. It binds to transcription factors such as Pol II to facilitate their transcriptional activity.

Interestingly, both Pol II and YAP demonstrated mixed patterns of movement within the nucleus. When they were not interacting with DNA or other proteins, they moved rapidly. However, when they bound to nuclear components at defined spatial locations, their movement slowed down.

Using so-SPIM-FCS, the scientists were able to record more than 3000 measurements for each protein, which allowed them to precisely predict protein dynamics across the 3D space of the nucleus. The capability of so-SPIM-FCS to produce clear images of subcellular structures along multiple planes, whilst minimizing phototoxic effects on cells under study, makes

it an ideal technique to track single protein dynamics inside the nucleus. This is displayed here, with the so-SPIM-FCS technique being used for the first time to unravel the dynamics of proteins such as YAP inside the nucleus.

The data from the so-SPIM-FCS measurements clearly highlights how protein activities constantly vary inside the nucleus, at various points in time and space. This is critical to understand how dynamic protein behavior influences nuclear organization, and as a result, controls cellular behavior at a global scale.

ABOUT THE RESEARCHERS: VIRGILE VIASNOFF



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TIMOTHY SAUNDERS



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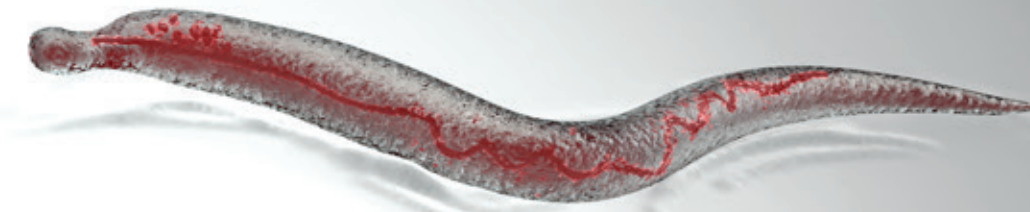
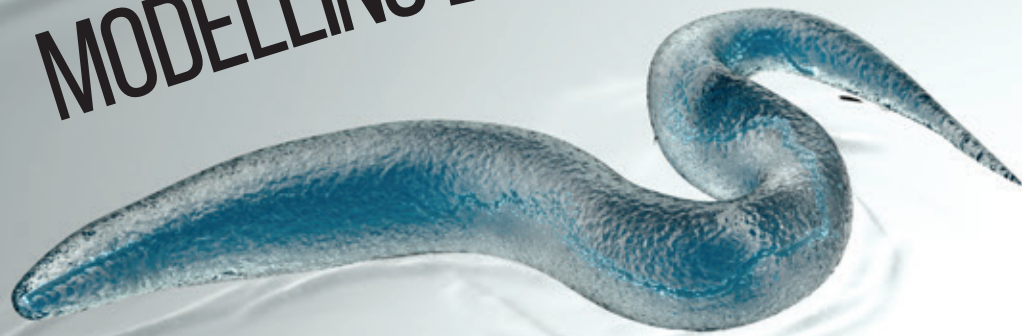
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MODELLING DEVELOPMENTAL DISORDERS

Written by Andrew Wong. Illustration by Diego Pitta de Araujo.



Collaborative research from MBI, National University of Singapore, and the University of Washington, Seattle, USA, has discovered that the AP-2 transcription factor is essential for regulating the timing of cell division during embryo formation. As mutations in AP-2 can lead to congenital birth defects, this study provides the first steps towards a mechanistic understanding of AP-2 related developmental disorders. This study is published in PLoS Genetics.

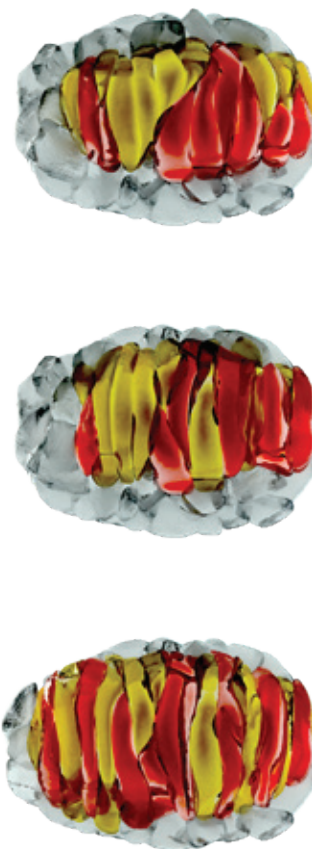
The development of a single fertilized egg cell into a multicellular embryo is a complicated, highly regulated process. In order to ensure a healthy embryo, a complex interplay of genetic instructions and mechanical forces must take place. While all the genetic information required for development is encoded in each cell, genes have to be switched on or off at specific times in order for development to proceed without problems. This is controlled by transcription factors, proteins which attach to DNA and act as genetic switches. The AP-2 family of transcription factors plays an important role in regulating cell division and cell death. Correct timing of both of these processes is essential throughout development. For example, incorrectly

timed cell division can lead to tumour formation, whilst programmed cell death is required during hand formation to create the gaps between fingers.

Mutations in the human AP-2 family are linked to two developmental disorders, Char syndrome and Branchio-oculo-facial syndrome (BOFS). Char syndrome affects the appearance of the face and hands, and also causes heart defects due to an inability to close a connection between the two major arteries. Branchio-oculo-facial syndrome manifests with abnormal patches of skin near the neck and ears, impaired vision, and facial defects such as a cleft lip. Although this family of transcription factors are known to be important in causing these diseases, the mechanism by which mutations in AP-2 leads to these two disorders remained unknown.

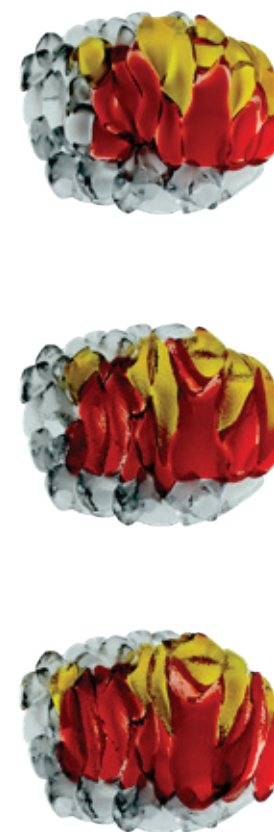
In order to tackle this question, a research team led by MBI Principal Investigator Asst. Prof. Ronen Zaidel-Bar used their expertise in genetics to identify and mutate the AP-2 gene equivalent in the nematode worm *C. elegans*. By observing the development of these mutated embryos, they discovered that a partial loss of AP-2 activity led to premature death of more

Normal embryos



As development progresses over time, epithelial cells in normal embryos intercalate by slotting in between each other in a defined order as illustrated by the red and yellow cells.

Mutant AP-2 deficient embryos



However, in AP-2 deficient mutant embryos, the epithelial cells fail to intercalate properly, leading to gap formation and cell leakage from the embryo.

than half of the embryos, and the few that remained alive had an abnormal shape. Observing these mutated embryos using 4D time-lapse microscopy revealed that they all suffered from major defects in their epidermal cells. In the absence of normal AP-2 activity, these skin cells were unable to arrange and slot together in the correct order, and gaps formed during embryonic development could not be sealed up, which occasionally led to cell leakage.

These results were validated by cell lineage analysis, where a cell is tagged with a fluorescent marker, allowing it and its progeny to be followed like a family tree. Examining the cell lineage tree from mutated embryos revealed a misregulation in the timing of cell division for determination of specific cell fates. Intriguingly, the pathways affected led to an absence of skin, muscle, and brain cells formation, the cell populations affected in BOFS and Char Syndrome.

Investigating congenital developmental disorders in humans can be difficult due to their rarity. This study shows how simple organisms can be used to model these diseases. Mutation of the AP-2 gene equivalent in the nematode worm

leads to embryonic defects that closely mirror the clinical manifestations seen in Char Syndrome and BOFS. Through the use of this model organism, it was discovered that misregulation of cell vdivision is a key mechanism responsible for causing these diseases, although there remains much more to uncover about these developmental disorders.

ABOUT THE RESEARCHER: RONEN ZAIDEL-BAR



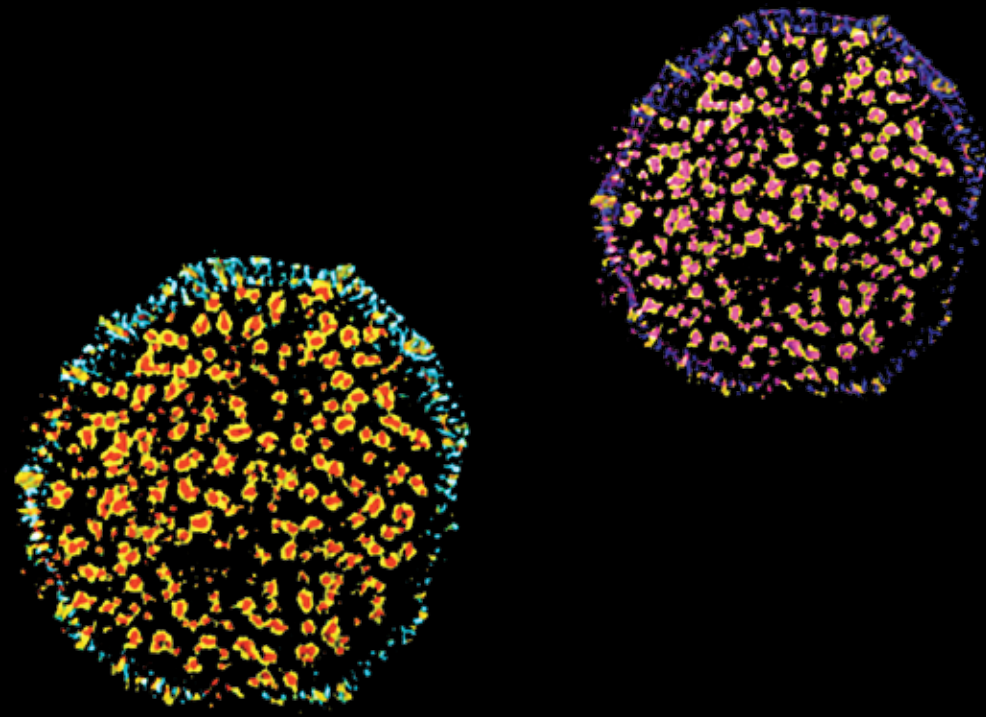
Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biomedical Engineering, NUS. The Zaidel-Bar

lab researches cell-cell and cell-matrix adhesion and the interplay of these forces with the actomyosin cytoskeleton.

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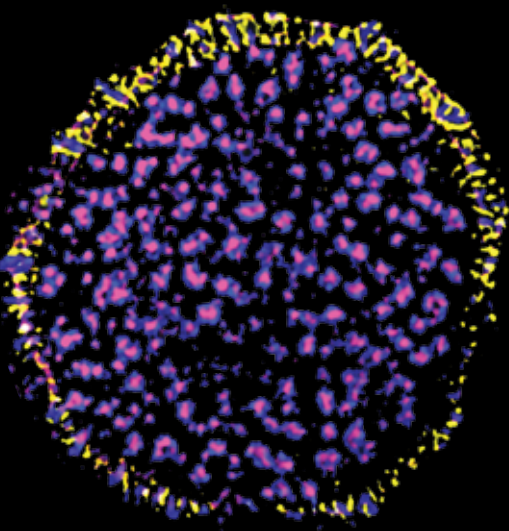
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CELLULAR PODIATRY

UNDERSTANDING HOW CELLS FORM FEET

Written by Lakshmi Ramachandran and Steven Wolf.



A study published in the Journal of Cell Biology has revealed that a protein known as Arf1 plays a role in podosome formation by regulating the assembly of myosin-II within the cytoskeleton. This study was carried out by a team of researchers from the labs of Prof. Alexander Bershadsky at the Mechanobiology Institute, National University of Singapore, and Prof. Gareth E Jones at King's College London. This work is published in the Journal of Cell Biology.

Molecular players in podosome formation

Some cells are constantly on the move. The cells of our immune system, for example, can only protect our bodies if they are able to track down potentially harmful bacteria or viruses. This means passing through or reaching deep inside the tissues and organs, to reach sites of infection.

To assist their movement through tissue, these cells have evolved structures that can be considered 'cellular feet'. Known as podosomes, these protrusions, of which there can be about a hundred per cell, make contact with a material that surrounds all cells (the extracellular matrix), and secrete proteins that degrade it. This action helps the moving cell to wade through tissue that is otherwise composed of impassable layers of matrix and tightly packed cells. In cancer cells, enlarged podosomes are often present (known as invadopodia) and these participate in the processes of cancer cell invasion and metastasis.

Like our own limbs, podosomes are composed of systems that physically support and facilitate their assembly, growth and movement. At the core of these cellular limbs is the cytoskeleton – a network of protein-based cables or filaments that provide structural support to cells, and at the same time are dynamically modified to produce the forces needed for cell movement. The main protein making up these filaments is known as actin.

Despite the importance of podosomes in immunity, the cellular mechanisms that control podosome formation were not clear. To better understand these mechanisms, Rafiq et al., employed super resolution microscopy to observe and describe the molecular steps that occur during podosome formation.

When podosome forming cells were viewed under super resolution microscopes, the podosomes could be seen as dots surrounded by a ring. These structures were found underneath the cell surface. The dot was found to correspond to a cytoskeleton core made of actin filaments. The ring around the actin core was made up of specialized proteins involved in the formation of cell-matrix contacts such as talin, paxillin and vinculin (Figure). Interestingly, viewing the cells in this manner also revealed a protein called Arf1, which serves as a switch to activate or deactivate cellular processes in response to signals coming from outside the cell. Previously, this molecular switch was believed only to control the transport of material within cells. Rafiq et al., however could now see Arf1 also co-localized with podosome rings.

Following this line of investigation, the researchers went on to discover that removal of Arf1 from cells rendered them incapable of forming podosomes. In this case Arf1 was controlling proteins involved in the formation of the cytoskeleton. One protein found to be directly impacted by Arf1's presence in podosomes was myosin-II, a protein that confers contractility to the cytoskeleton. Importantly, myosin-II is not found in the cytoskeletal core of the podosome, however it is found at the periphery. When Arf1 was reduced, myosin-II increased, suggesting this molecular switch controls how much myosin is present in the area, as too much would prevent podosome formation.

From this study Rafiq et al., speculate that Arf1 regulates the formation of podosomes by repressing the activity of myosin-II in the cytoskeleton. The roles of Arf1 and myosin-II in podosome formation, which were previously unknown, provide valuable insights in our understanding of how cells, including those of the immune system, move through tissue. With larger podosome-like structures often found in cancer cells, such knowledge also sheds light on how and why some cells acquire the ability to spread around the body, invade tissues and form tumors. By targeting the Arf1 it is hoped that processes of cell migration, invasion and matrix degradation, which are all crucial steps in the onset of cancer, can be modulated.

Figure: The images show actin cores and vinculin rings of podosomes, as well as localization of myosin-IIA filaments (cell edge) in TGFβ1-stimulated macrophage-like THP1 cell visualized by structured illumination microscopy (top). These myosin-IIA filaments are absent at sites of podosome assemblies, but are localized to actin stress fiber-like structures upon treatment with the ARF inhibitor, SecinH3 (bottom). Actin (blue) was stained by phalloidin, while vinculin (red) and myosin-IIA heavy chain (yellow) were labeled with corresponding antibodies.

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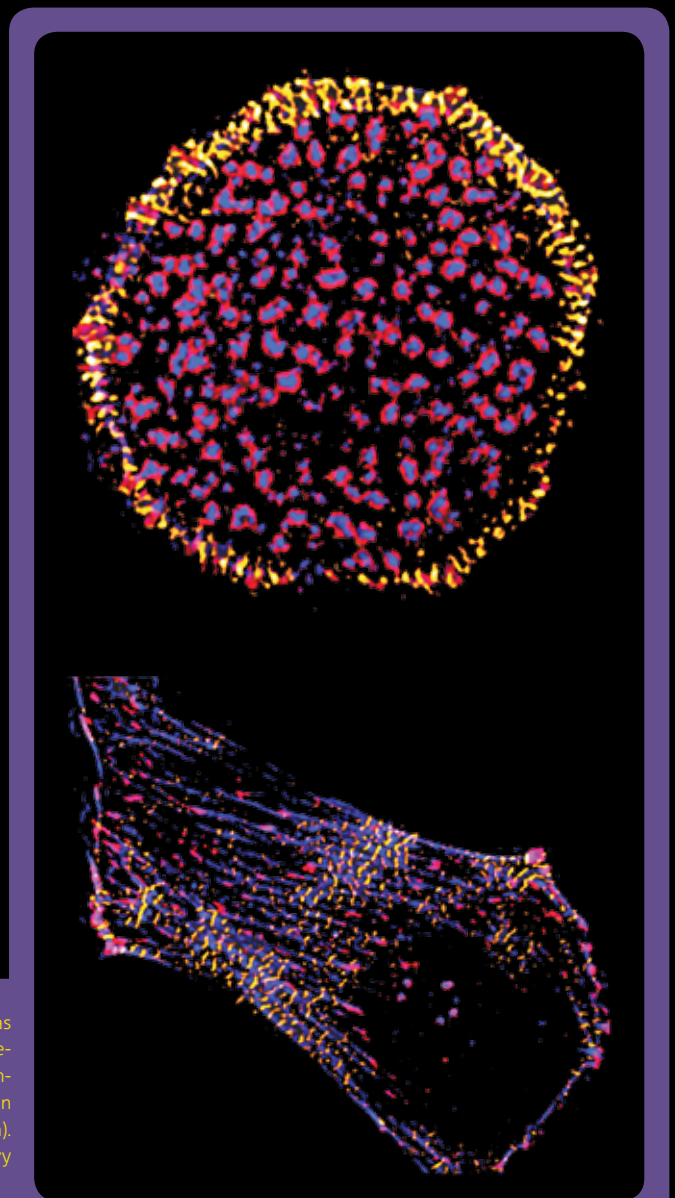


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BAILED OUT

Written by Sruthi Jagannathan. Illustration by Diego Pitta de Araujo.

A multi-disciplinary research team led by investigators from MBI, National University of Singapore, along with colleagues from the Institute of Bioengineering and Nanotechnology (IBN) of A*STAR, and BioSyM, Singapore-MIT Alliance for Research and Technology have described the mechanical principles adopted by liver cells as they remove excess bile during obstructive cholestasis. This study was published in the *Journal of Hepatology*.

ABOUT THE RESEARCHERS:

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Biliary atresia is a rare, life-threatening liver disease that affects infants, and in particular, babies of Asian and African American descent. Although the cause of the disease remains unclear, the first symptoms are usually detected within weeks of birth.

Dubbed the 'chemical factory' of our body, the liver performs over 500 biological functions. Foremost among them is the synthesis and storage of essential biochemical substances, as well as the elimination or neutralisation of harmful toxins from the blood.

One of the most important functions of the liver is the production of bile, which is responsible for the organ's detoxifying effects. Bile flows through a network of tubes known as the biliary tract, into the small intestine. During its passage the bile digests fats, and absorbs essential fatty acids. It also facilitates the elimination of waste products via the faeces.

In babies suffering from biliary atresia, bile accumulates in the biliary ducts. This means that liver function is ultimately impeded and without surgical intervention, long-term liver damage or cirrhosis will occur.

To date there are no drugs available to treat biliary atresia. However, there is renewed hope that this may one day change, with recent findings from research conducted at MBI revealing how liver cells already possess the ability to eliminate excess bile from tubes located inside the liver, which feed bile into the biliary ducts.

How liver cells eliminate bile from blocked ducts

The key to the liver's ability to eliminate excess bile is the fact that the 'tubes' through which bile enters the biliary tract are not merely a set of inactive pipes, but are actually hollow spaces between living cells. The walls of the tubes are essentially the outside surfaces of the cells.

To investigate how the liver responds to bile accumulation, the research team used an artificial culture system, which allowed the easy manipulation of cultured liver cells. They then used high-end imaging techniques to visualise the dynamics of the tubes that develop within this culture system.

The team sought to investigate the response of the cells that line a blocked bile duct by obstructing the bile tract artificially and observing what happened. What they found was that as the bile accumulated behind the blockage, the tube began to swell or bulge, and this put pressure on the cells that make up the wall of the tube.

The key to the removal of excess bile lies in the internal structure of the cell itself. Immediately adjacent to the cell membrane is a network of protein cables or filaments known as the actin cortex. This structure serves to strengthen the cell, and help it retain its shape and integrity even when external forces are applied to the cell surface - external forces like the increased pressure from a build-up of fluid. Normally the actin cortex is able to counter the forces applied to it, and even when some

damage to the network is incurred, it is quickly fixed by proteins that can reassemble the actin filaments.

However, when the pressure becomes too great, the actin cortex will rupture, and it will not be repaired. Although the bile cannot simply pass through the membrane, it can, as the researchers discovered, push the membrane into the cell, through the gap in the ruptured actin cortex. As this occurs a bubble-like vesicle forms inside the cell, and it is inside this vesicle that the bile enters and passes through the cell. The bile is essentially packaged inside these vesicles for its transport through the cell, and away from the site where it had accumulated.

Although the liver does not stop producing bile even when the biliary ducts are blocked, it is now evident that the liver does have a process in place to look after itself when a potentially damaging amount of bile builds up inside a blocked duct.

It is hoped that this mechanism may one day be therapeutically targeted to improve the prognosis for infants with biliary atresia. As the researchers showed, the rate of vesicle formation, and hence the uptake of excess bile into liver cells, can indeed be adjusted using drugs, at least in the cell culture setting. Improving the effectiveness of this naturally occurring mechanism, by increasing, for example, the rate of vesicle formation, may indeed encourage bile elimination from the blocked duct so as to avoid long-term liver damage and increase the effectiveness of surgical intervention.

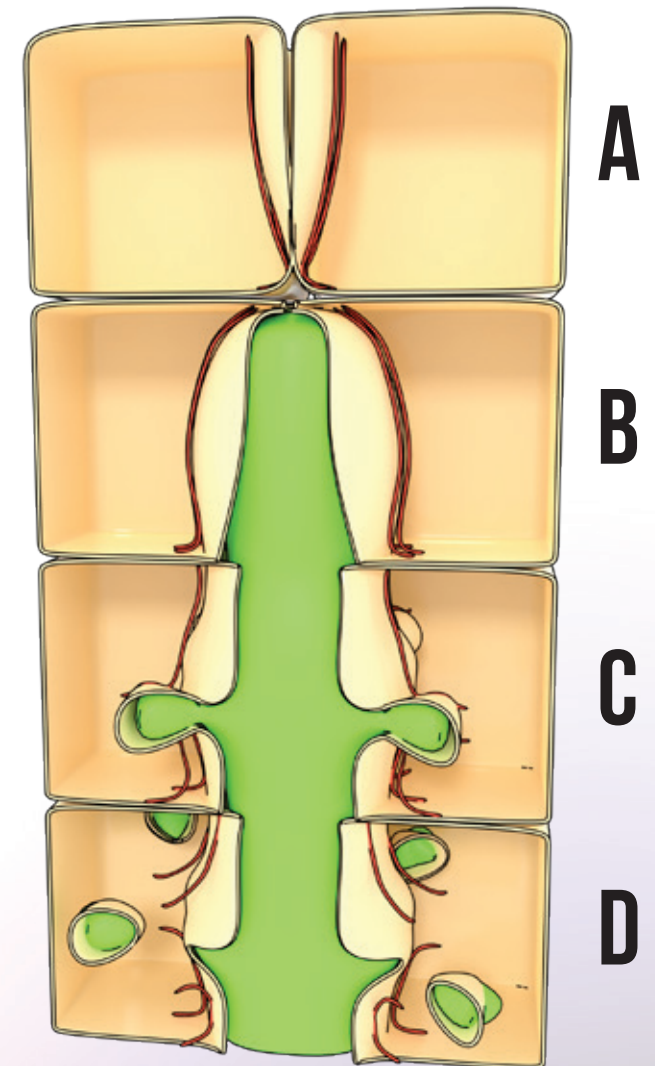


Figure: Schematic diagram illustrating how hepatocytes or liver cells clear excess bile from blocked ducts. (A) Bile ducts are blocked in diseases such as biliary atresia. (B) Bile builds up behind the blockage, putting pressure on the surrounding cells that make up the walls of the duct. (C) If the pressure increases, the actin cortex, which lies inside the cell, may rupture. Once the actin filament network is fragmented, the bile will push the cell membrane into the cell, through the disrupted actin network. (D) If the actin network cannot be repaired, then the membrane will continue to be pushed inside until it breaks off into bubble-like vesicles. These vesicles carry the bile through the cell and away from the blockage.

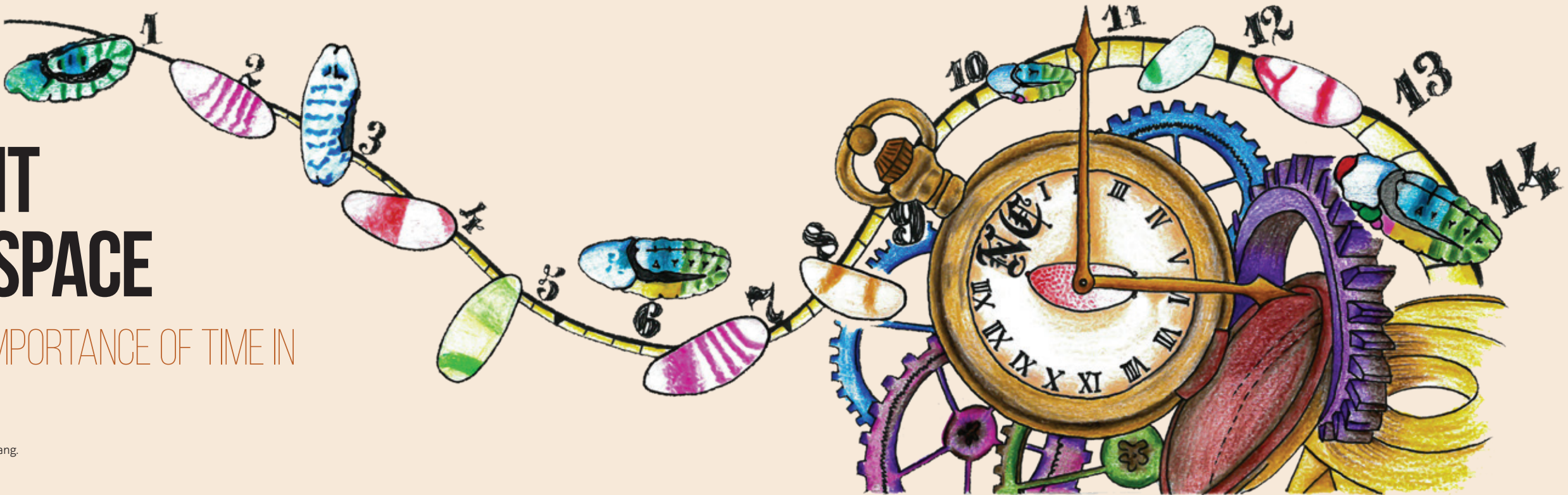
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DEVELOPMENT IN TIME AND SPACE

SHEDDING LIGHT ON THE IMPORTANCE OF TIME IN EMBRYO DEVELOPMENT

Written by Andrew Wong and Steven Wolf. Illustration by Anqi Huang.



Researchers from the Saunders lab at MBI, National University of Singapore have used optogenetics to reveal how protein concentration gradients impart both spatial and temporal information for the determination of cell fate during development. This study is published in eLife.

Biological organisms display a range of patterns and spatial features, from leopard spots to the stripes on fish. Understanding how these patterns emerge at the correct time and place is a major problem in biology. During development cells need to take on specific cell fates. How, where, and when cell fate is determined has been

the source of intense study. Alan Turing first posited the idea of a **morphogen**, a signalling molecule that originates from a single cell before spreading through a tissue, thereby establishing a morphogen gradient, in his seminal 1952 paper “*The Chemical Basis of Morphogenesis*”. He demonstrated that a wide range of biologically relevant patterns can emerge from surprisingly simple networks of only two morphogens. More recently, it has been demonstrated that embryos can interpret morphogen gradients with remarkable precision, enabling robust embryonic development.

The key idea behind the morphogen hypothesis is that certain genes can

be “switched on” or “off” depending on the concentration of morphogen input that the cell is exposed to. This input concentration is dependent on **where** the cell is relative to the morphogen source. However, recent work has revealed that ‘temporal’ or time-based information is also utilised by biological systems – i.e. **when** the signal is received and interpreted. For example, how long a cell is exposed to a particular morphogen, or whether the signal is detected at a specific time in the sequence of developmental events, can play a role in cell fate determination.

Networks that allow cells to robustly interpret morphogen signals have now

been found in a variety of systems, from the early *Drosophila* (fruit fly) embryo to the development of the vertebrate nerve cord. The first experimentally discovered morphogen is Bicoid, which is a transcription factor that binds to DNA, and forms a gradient in the early *Drosophila* embryo. The concentration of Bicoid is highest at the anterior pole (front) of the embryo, and decays as it spreads towards the posterior pole (rear).

It has been shown that spatial information from the Bicoid gradient is important in determining the fate of cells in the developing fly embryo. However, determining the influence of temporal information, such as the length of time in which cells are exposed to Bicoid, has been particularly challenging.


In order to address this problem, MBI researchers Anqi Huang and Christopher Amourda from the lab of Asst. Prof Timothy Saunders utilised a method known as optogenetics, to control Bicoid signalling in the developing fruit fly embryo. Optogenetics uses light activation to alter the configuration of light-sensitive proteins. The Saunders lab fused such a light-sensitive protein

to Bicoid. When embryos expressing the light-sensitive Bicoid fusion were kept in the dark, Bicoid activity and development proceeded as normal. However, upon activation by blue light, the light-sensitive Bicoid fusion could no longer support transcription, effectively turning off the signalling pathways that depend on Bicoid input. Importantly, the concentration gradient of Bicoid itself was left untouched.

With the ability to precisely control the temporal component of gene activation inside the living embryo, the researchers were able to determine the importance of the timing and duration of Bicoid signalling. Interestingly, switching off Bicoid in the first hour after fertilization did not affect the embryo, but after this stage, interrupting Bicoid activity led to problems or failure in embryonic development. In addition, cells located in the embryo anterior (which are exposed to high concentration of Bicoid), also needed to be exposed to Bicoid for a longer period in order to undergo specialization. Conversely, cells located at the embryo posterior, where Bicoid expression is low, only required a short period of Bicoid exposure before they became specialized.

The importance of time in embryo development has been made clearer from this work. How long cells are exposed to Bicoid, and not just how much Bicoid they are exposed to, impacts cell fate determination. The use of optogenetics to dissect both the spatial and temporal aspects of morphogen gradients is now possible, and promises to unlock the temporal dimension of other similar signalling systems in the future.

ABOUT THE RESEARCHER: TIMOTHY SAUNDERS

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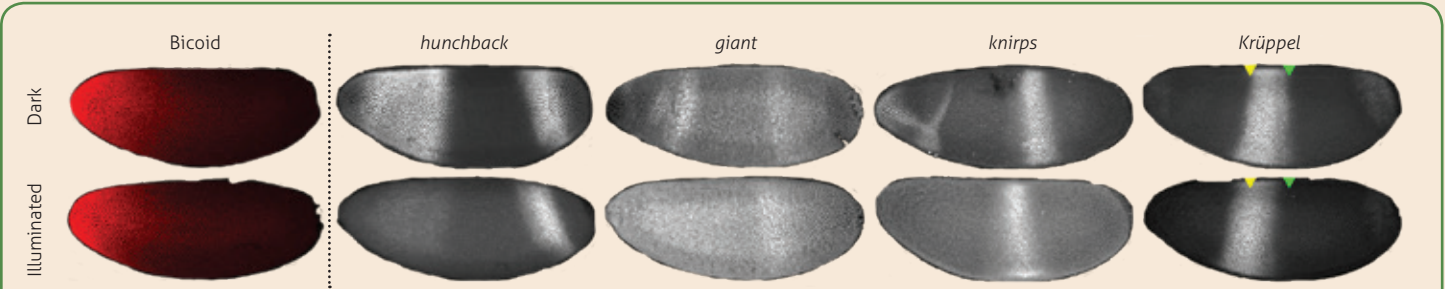


Figure: *Drosophila* embryos expressing the optogenetic Bicoid cassette developed either in the dark or under illuminated light. The anterior-posterior Bicoid concentration gradient is unaffected by the optogenetic cassette. Embryos were fixed at the end of the blastoderm stage and stained for developmental gap genes *hunchback*, *giant*, *knirps* and *Krüppel*. Illuminated embryos exhibit reduced gene expression of anterior *hunchback*, *giant*, and *knirps*, while their posterior expression remains intact. The *Krüppel* band shifts towards the anterior pole in illuminated embryos compared to in the dark. Yellow and green arrowheads indicate, respectively, the anterior and posterior Kr boundaries in the dark. Adapted from Fig 1, Huang A et al., *eLife* 2017;6:e26258

CELL EXTRUSION MECHANISMS

Written by Andrew Wong. Illustration by Diego Pitta de Araujo.



An international collaboration between scientists from the Mechanobiology Institute, National University of Singapore, and Institut Jacques Monod & Université Paris Diderot, France, has revealed how epithelial cell extrusion is regulated by cell density. This study is published in *Current Biology*.

How does cell density affect extrusion?

The external and internal surfaces of the body are covered by a layer of cells known as epithelial cell sheets. The classic example of an epithelial cell sheet is skin, but epithelial layers also line internal cavities such as blood vessels, the stomach, and the mouth. The primary role of these cell sheets is to provide a protective barrier against physical damage and infection. In order to perform these functions, the integrity of the epithelial cell sheet must be maintained by balancing cell renewal and removal. For example, the layer of cells lining the intestine is renewed every 5 days. Deteriorating, damaged, or unnecessary cells are targeted for elimination by apoptosis – the process of programmed cell death – allowing them to be eliminated without causing damage to the neighboring healthy cells, as would occur during inflammation.

Removal of these apoptotic cells from the epithelial cell sheet to maintain an intact

barrier layer takes place by the process of cell extrusion. To date, studies have shown that epithelial cell extrusion occurs via formation of a contractile ring made up of protein based cables and motors in the cells surrounding the cell targeted for extrusion. The contractile ring tightens around the base of the extruding cell, pushing it out of the epithelial sheet and bringing the surrounding cells together.

Although this 'purse-string' mechanism of contraction is commonly seen in epithelial cell sheets, many of these observations have been based on the assumption that the epithelial layer is a collection of individual cells. However, in reality, these multi-cellular sheets are highly complex structures, with large variations in cell dynamics and cell density.

In order to account for this level of complexity, an interdisciplinary team of biologists, engineers, and biophysicists was assembled by Prof. Benoit Ladoux from MBI and Institut Jacques Monod, France and Asst. Prof. Yusuke Toyama from MBI. The scientists used microfabrication to create circular micro-patterned surfaces that enabled control of the growth and density of epithelial cell sheets. By observing cell extrusion events in cell sheets grown on these patterns, with time-lapse and traction-force microscopy, they discovered that cell density led to two distinct modes of cell extrusion. At a low cell density (above illustration, blue-green cells), the cells in a

tissue are dynamic and mobile. As these cells are moving freely, occasionally cell density becomes high in a small patch in the tissue. Cells at this dense region undergo apoptosis, and the cells surrounding the apoptotic cell selected for extrusion collectively crawl towards the targeted cell, and extend large, flat protrusions called lamellipodia underneath it. This action levers the apoptotic cell out of the sheet, causing its extrusion.

However, at high density (above illustration, yellow-gold cells) cells are too tightly packed to move, preventing collective cell migration and lamellipodia-based extrusion. Under these conditions, the cells surrounding the apoptotic cell form a contractile ring (above illustration, red ring), and use purse-string contraction to squeeze out and extrude the cell.

This study revealed, for the first time, that two distinct mechanisms exist to expel apoptotic cells from epithelial cell sheets. Selection between cell extrusion mechanisms is defined by cell density – cell crawling and lamellipodia extension is the predominant mechanism at low density, but purse-string contraction is favoured at high density. The existence of these complementary mechanisms could be important for ensuring the removal of unnecessary cells (e.g., apoptotic cells) in different circumstances to maintain the integrity of the epithelial cell sheet.

ABOUT THE RESEARCHER: BENOIT LADOUX



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Kocgozlu et al., Epithelial cell packing induces distinct modes of cell extrusions. *Curr. Biol.* 2016, <http://dx.doi.org/10.1016/j.cub.2016.08.057>

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MBI is also recruiting for research assistants to be assigned to research groups at the Institute. Candidates are to work under a supervisor and undertake both research administration, laboratory management as well as research work. We welcome applications from degree holders in Science or Engineering with a preference for candidates with some education in Life Sciences.



For current vacancies at MBI, please visit: mbi.nus.edu.sg/category/jobs/ or email: mbihr@nus.edu.sg

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