October 2016

MBINSIGHTS

DECODING THE LIVING MACHINE

The biophysics of Salmonella infection

Advances in super-resolution microscopy

Actin shields the nucleus against distant forces

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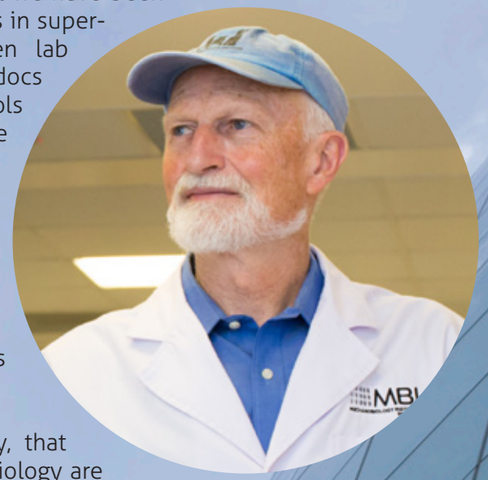
FROM THE DIRECTOR



The purpose of these synopses is to show the breadth of the research coming from the MBI. We are very proud of the fact that we have been able to build an institute with state-of-the-art capabilities in super-resolution microscopy and microfabrication. Our open lab environment and the energy of our students and postdocs have combined to take advantage of our experimental tools to answer important biological questions. Because we believe that these tools should be utilized to solve many more problems in the biomedical sciences, we invite you and your colleagues to join us in collaborative studies to solve those problems. Many different disciplines are needed since the problems need the tools of physicists, biologists, computational scientists, and engineers, working side-by-side to better understand how biological systems integrate mechanical cues and physical forces from the world around them.

Such is the interdisciplinary nature of Mechanobiology, that problems traditionally in the realm of cell or molecular biology are now being tackled by physicists and theorists. By quantitatively assessing what previously could only be observed, and simulating what is otherwise speculative, we have been able to answer an array of questions on how biological systems develop in physically distinct environments, and why things go awry and disease states arise, when the environment changes.

This is most clearly reflected in the research from an interdisciplinary environment. In 2015 and 2016, we saw numerous high impact studies come to fruition, and these research highlights are compiled here, in our inaugural research magazine, MBInsights.

On the cover is an artistic impression of how an actin network will form around the nucleus in response to a physical, yet distant impact, at the cell membrane. We have also described how the cell cytoskeleton forms with a distinct handedness, or chirality, and have explored how some microbes not only survive within immune cells, but use the acidic environment of a vacuole to their advantage. Novel methods for culturing harvested cancer cells, and an innovative method to attain super-resolution quality imaging from standard inverted microscopes, are also described in this issue.

I hope that you find these research summaries interesting, and we invite you to join us in collaborative studies at the MBI.

Professor Michael P. Sheetz,

Director
Mechanobiology Institute, National University of Singapore

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.

MBI
MECHANOBIOLOGY INSTITUTE
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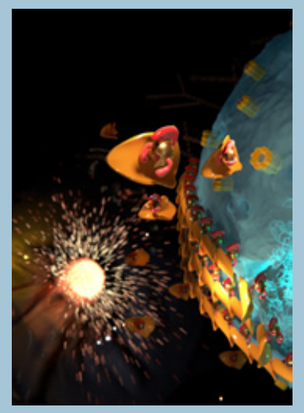

About the Cover:

The cover depicts the formation of the actin rim around the nucleus in response to a distant force, which may act as a shield to protect DNA from mechanical forces and stabilize nuclear functions. For more details, please read the feature "Forces Acting Far and Wide" on pages 14-15.

Cover illustration by Diego Pitta de Araujo.

MBINSIGHTS
DECODING THE LIVING MACHINE

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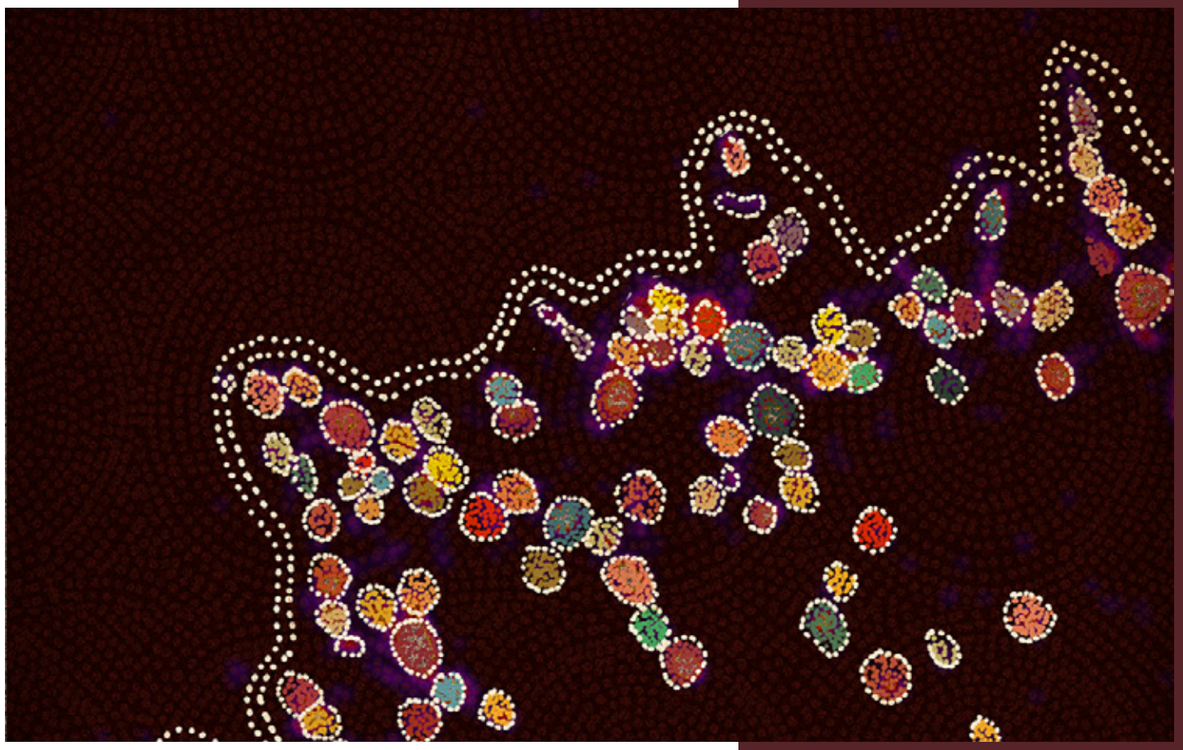
MBInfo

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!



Artistic impression, inspired by Australian Aboriginal art, of the clustering of integrin-based adhesion complexes. Artwork by Rishita Changede.

ADHESION ABC

INTEGRIN CLUSTERS ARE THE UNIVERSAL UNITS OF CELL ADHESION

Written by Andrew Wong.

Published in Developmental Cell, a study led by Senior Research Fellow Dr. Rishita Changede, discovered the universal building blocks that cells use to form initial connections with the surrounding environment. These early adhesions have a consistent size of 100 nanometres and are made up of a cluster of around 50 integrin proteins, and are the same even when the surrounding surface is hard or soft. Deciphering the universal nature of adhesion formation may reveal how tumour cells sense and migrate on surfaces of different rigidity, which is a hallmark of metastasis, the devastating ability of cancer to spread throughout the body.

Building blocks for cell adhesion

Cells interact with their environment through protein complexes called focal adhesions. These act as the hands and feet of the cell, and allow it to form physical connections with the surrounding surface, enabling the sending and receiving of mechanical signals from the environment. This, in turn, drives many of the cell's decisions, such as determining what type of cell to develop into, or where in the body it should move to. However, within the body there are a variety of different surfaces that cells can grow on, from soft surfaces such as brain matter to hard surfaces like bone. Although cells can form focal adhesions on both soft and hard surfaces, how they assemble on surfaces of such different rigidity is still a mystery.

The major protein in the focal adhesion complex is integrin, which spans the cell membrane, forming a link between the internal skeleton of the cell and external surface. Integrin binds to a sequence of three amino acids (arginine-glycine-aspartic acid, also known as RGD). By growing cells on either hard glass or fluid artificial membranes coated with RGD, and observing adhesion formation with super-resolution microscopy, a team of MBI scientists led by Senior Research Fellow Dr. Rishita Changede and Prof. Michael Sheetz were able to investigate the molecular origins of adhesion formation on surfaces with different rigidity. Analysing this data with custom-built computational algorithms allowed them to accurately measure adhesion size, and even count the number of integrins in each adhesion.

Remarkably, they discovered that cells growing on soft or hard surfaces form adhesions in the exact same way. These early, or nascent adhesions assemble from clusters of approximately 50 integrins, and have a diameter of around 100 nanometres. Despite the small size of early adhesions compared to the average cell surface area of 3,500 square micrometres, they enable the cell to form an initial attachment to the environment. Intriguingly, altering the density of the RGD coating also had no effect on the formation of early adhesions. The same integrin clusters were formed, with a consistent protein composition and size, even when the RGD density was reduced 10-fold.

As the early adhesions formed independently of surface rigidity or RGD density, they represent universal, modular units for cell adhesion to the environment. Similar to building blocks, these modular units could aggregate together to form larger adhesions. Formation of early adhesions was also assisted by rapid recruitment of the mechanosensor protein, talin. Once the cell forms these early adhesions on soft or hard surfaces, it can use talin and other mechanosensitive proteins to develop force. Depending on the force received from the surface, the early adhesions can either recycle back into the cell, or mature into focal adhesions.

This study revealed that cells form early adhesions from integrin clusters as a first response to their environment, and that these universal, modular units of adhesion assemble without the need for external stimuli from the surrounding surface.

Understanding how these universal early adhesions form the building blocks for mature focal adhesions will provide new insights into focal adhesion mediated mechanosignalling and its vital role in cell growth, development, and disease.

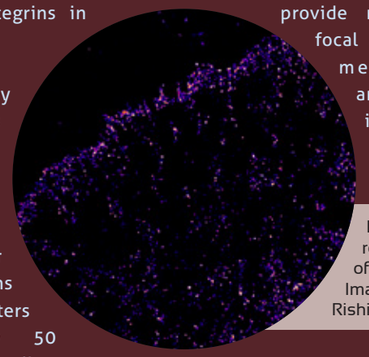


Figure: Super resolution image of integrin clusters. Image courtesy of Rishita Changede.

REFERENCE:
Changede R, et al. Nascent Integrin Adhesions Form on All Matrix Rigidities after Integrin Activation. Developmental Cell. 2015. 35(5):614-21. doi: 10.1016/j.devcel.2015.11.001.

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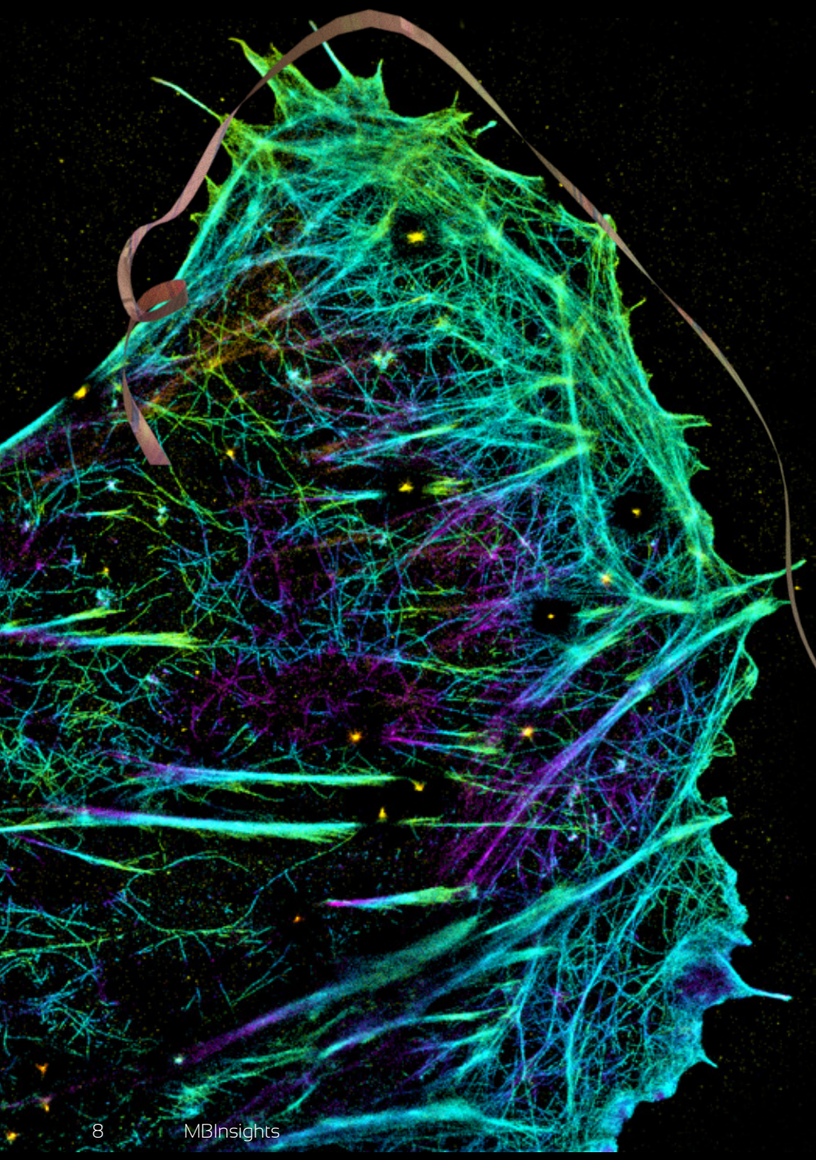
DISSECTING FOCAL ADHESIONS

Written by Lakshmi Ramachandran. Image by Diego Pitta de Araujo.

For years biology was studied independently of physics. Despite this, cells and other biological systems live under the influence of physical forces. Physical properties of the environment can define how cells move, grow and form tissues or organs. Cells can also generate their own forces, and do so with such precision that specific processes like tissue formation repeatedly give rise to organisms with well-defined sizes, shapes and appearance. The influence of physics on biological phenomena has presented scientists with a new paradigm in which to understand life. One of the main questions being asked now is what lies inside cells that allow them to respond to the physical world around them. How do cells know when to move, and where to move to? How can cells avoid being damaged by forces that are too strong or too weak to support them?

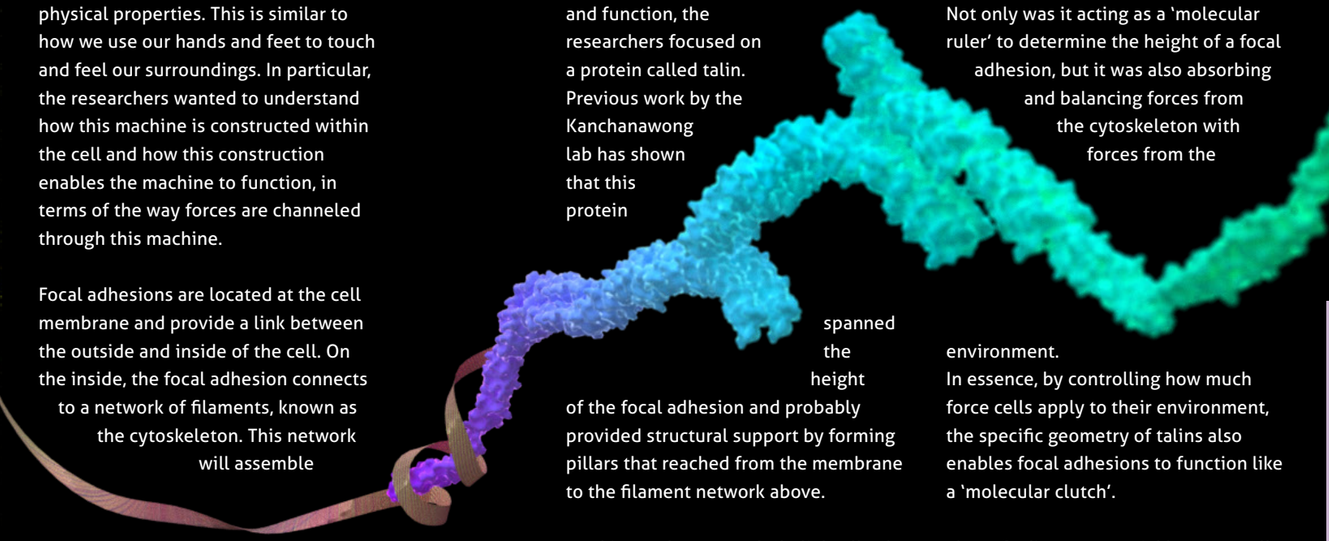


Reverse engineering molecular machines

Following this line of enquiry, a group of scientists led by MBI Principal Investigator Asst. Prof. Pakorn (Tony) Kanchanawong, focused on picking apart a molecular machine called focal adhesions, which cells use to attach to their environment and measure its physical properties. This is similar to how we use our hands and feet to touch and feel our surroundings. In particular, the researchers wanted to understand how this machine is constructed within the cell and how this construction enables the machine to function, in terms of the way forces are channeled through this machine.

Focal adhesions are located at the cell membrane and provide a link between the outside and inside of the cell. On the inside, the focal adhesion connects to a network of filaments, known as the cytoskeleton. This network will assemble and disassemble according to whether the cell needs structural support, or needs to move. On the outside, the focal adhesion connects to specific proteins in the immediate environment, called matrix proteins. Interestingly, like a clutch that controls the amount of power transmitted from the engines to the wheels of a car, focal adhesions can actively adjust how much force cells apply to their environment. However, the changes that occur at the molecular level have remained unclear.

With an aim to understand how the size or geometry of particular protein components within focal adhesions could affect their architecture and function, the researchers focused on a protein called talin. Previous work by the Kanchanawong lab has shown that this protein spanned the height of the focal adhesion and probably provided structural support by forming pillars that reached from the membrane to the filament network above.



Working on human endothelial cells, the group replaced naturally occurring talin with a set of artificially synthesized talin proteins of varying lengths. This was achieved using protein engineering, where distinct segments of the protein chain were removed, thus producing progressively shorter yet functional proteins. Using super-resolution microscopy to actually see the effect of providing cells with talin molecules that ranged in length, the group revealed just how significant these individual components are in the function of the focal adhesion.

Molecular rulers of focal adhesions

As talins of decreasing lengths were introduced, focal adhesions became shorter, showing that it was talin that was determining the height of the focal adhesion. In terms of geometrical orientation, talins were found to occur diagonally in the focal adhesion, which led the researchers to identify additional roles for the protein.

Not only was it acting as a 'molecular ruler' to determine the height of a focal adhesion, but it was also absorbing and balancing forces from the cytoskeleton with forces from the environment.

In essence, by controlling how much force cells apply to their environment, the specific geometry of talins also enables focal adhesions to function like a 'molecular clutch'.

These findings are crucial because cells in our bodies are constantly under stress from physical forces. To ensure cells can form and maintain tissue organization, they require molecular machines to absorb and transmit forces, or even generate counter forces. Only now are scientists beginning to understand the significance of the relationship between physics and biology in the development of complex organisms. In this study, super-resolution microscopy has been the essential tool that allowed researchers to see individual components of the focal adhesion and determine their function.

With super-resolution microscopy now becoming more widely available, an exciting time lies ahead for science as researchers better understand the engineering principles that cells make use of and how physical phenomena is integrated into biological systems to give rise to the life that we see and feel around us every day.

Nanoscale Architecture

iPALM 3D super-resolution microscope image showing the network of filaments at a focal adhesion.

Image provided by Dr. Wang Yilin, from the lab of Pakorn Kanchanawong.

ABOUT THE RESEARCHER:

PAKORN TONY KANCHANAWONG

Principal Investigator at the Mechanobiology Institute, and Assistant Professor at the Department of Biomedical Engineering, NUS. The scientific interest of his lab is at the interface of cell biology, biophysics, and advanced imaging technology.

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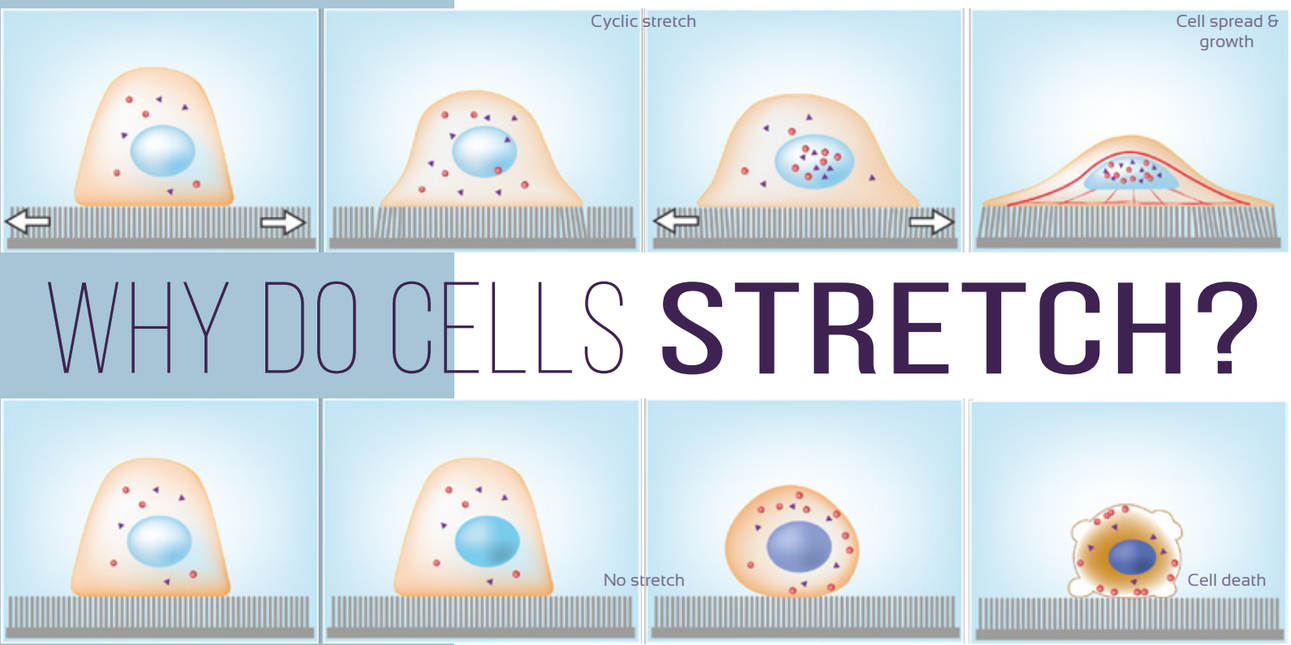
REFERENCE:
Liu J et al. Talin determines the nanoscale architecture of focal adhesions. Proc Natl Acad Sci USA. 2015 doi: 10.1073/pnas.1512025112



WHY DO CELLS **STRETCH?**

Written by Andrew Wong. Illustration by Cindy Zhang.

A research team, led by MBI Director Prof. Michael Sheetz, determined how soft tissues use cycles of mechanical stretching and relaxation for essential cell functions, including growth. This relates to the problem of soft tissue loss with disuse. This study was published in Nature Communications.

Surfaces change cell behaviour

Any runner will tell you that running on a sandy beach is much more challenging than running on tarmac. The limited resistance from the sand means that your feet sink into the soft surface with every stride. Running successfully on sand requires you to continually adapt to the changing surface, so you end up burning more energy to maintain balance and coordination.

Similarly, the cells in your body react differently when they are growing on a hard supporting layer, as opposed to on a soft one. The principle underlying this is *mechanotransduction*, where cells sense mechanical stimuli and convert them into biochemical signals. Cells are constantly pulling on the surface they grow on to test its stiffness, and react by adjusting their shape and stickiness. For example, when cells grow on a hard surface such as bone they flatten and spread out, forming long, stable edges. These spread out cells develop stress fibres, which physically connect the skeleton of the cell to the surrounding environment, to facilitate mechanotransduction.

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. His 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:

Cui et al., Cyclic stretching of soft substrates induces spreading and growth, Nature Communications, 23 Feb 2015, doi: 10.1038/ncomms7333

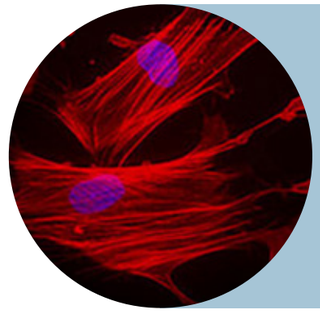
In the case of tissues like skin and lungs, cells are surrounded by soft surfaces. Without physical resistance from the environment, these cells do not receive the mechanical signals that they need for vital biological functions, including cell migration, growth and differentiation. However, soft tissues can undergo cycles of stretching and relaxation in the body, for example, inflation and deflation of the lungs while breathing.

By combining cell culture with nanofabrication, a team of MBI researchers led by Prof. Michael Sheetz discovered that this cyclic stretching generates mechanical stimuli which mimics the resistance from a hard surface. Cells were grown on a nanofabricated sheet of soft pillars, where each pillar had a diameter of 500nm, more than 200 times smaller than the average width of human hair. These nanoscale pillars mimic a soft surface, with the added ability to be stretched in all directions. Without any pillar stretching, cells did not spread, form stress fibres, or grow. Even when the pillars were stretched once, the cells remained rounded and unable to spread.

However, when these pillars were repeatedly stretched and relaxed, the cells spread out and developed stress fibres as if they were growing on a hard surface. The mechanical stimuli from cyclic stretching also caused biochemical changes, with DNA binding proteins moving to the nucleus, leading to increased cell division. Remarkably, cyclic stretching of the pillars by just 1% was sufficient to stimulate cell spread and growth. This study showed that cyclic stretching of the supporting layer provides an essential mechanical stimulus for cell growth on soft surfaces.

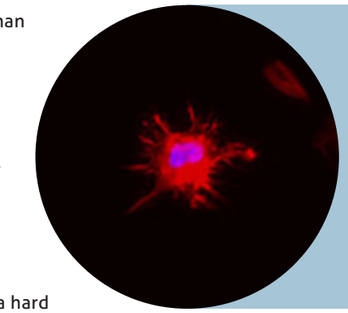
Soft tissues rely on cycles of mechanical stretching and relaxation to spread and grow. Understanding these mechanical cues will be essential for tissue and organ engineering, and for combating age related tissue loss

Fortunately, many of our soft tissues such as blood vessels and lungs already undergo these small, regular mechanical distortions during normal life activities of walking and breathing. However, for other soft tissues, especially skin and muscles, it is important to exert some physical activity to keep these cells functioning properly. This is particularly relevant for the ageing population, as the matrix surrounding our tissues degrades and becomes softer as we get older. As well as aiding in combating the ageing process, understanding the mechanical cues necessary for soft tissue function will be essential for tissue and organ engineering.



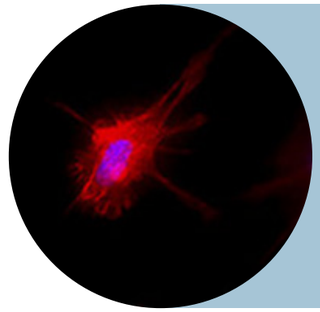
Hard surface

On a stiff, flat surface, cells spread out, grow, and form stress fibres (red actin filaments).



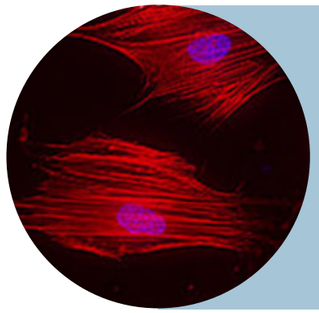
No stretching

When grown on a surface of soft pillars that is not stretched, cells do not spread or grow, and eventually round up and die.



Static stretch

A single stretch of the pillars enables some cell growth but no stress fibre formation.



Cyclic stretching

Repeated, cyclic stretching of the pillars results in healthy, spread cells which are identical to cells grown on a hard surface.

REGULATING NEURON GROWTH

Written by Lakshmi Ramachandran. Illustration by Diego Pitta de Araujo.

MBI researchers, led by Assoc. Prof. Boon Chuan Low, discovered the molecular mechanism behind neuronal signaling via the neurotransmitter acetylcholine (ACh), which is critical for brain function and development. This work was published in Developmental Cell.

Our body is wired by a network of nerves that transmit information in the form of electric signals to and from the brain, eventually dictating all of our actions. Key to this transmission of nerve impulses are a class of chemicals commonly referred to as neurotransmitters. Often found at synapses, which are the junctions between individual nerve cells or neurons, neurotransmitters relay information from one neuron to another. There are different types of neurotransmitters performing a variety of roles, ranging from controlling muscle movement, memory and even our mood.

The first neurotransmitter discovered, acetylcholine, is required for critical functions like muscle stimulation, learning and memory. Therefore a drop in ACh levels is, not surprisingly, associated with movement disorders like paralysis, as well as neurological diseases such as dementia and Alzheimer's disease. From extensive research on ACh, we know that at the molecular level, it promotes the development of nerve cells through a process known as neurite outgrowth. During this process, multiple dendrites and a single long axon begin to project out from the nerve cell body, giving the neuron its typical shape.

However, the spatial regulation of ACh synthesis remained unclear until Jichao Sun, a postdoctoral researcher from the lab of Assoc. Prof. Boon Chuan Low discovered that a protein called BNIP-H regulates the precise localization of the metabolic enzymes ATP citrate lyase (ACL) and choline acetyltransferase (ChAT), which are responsible for ACh synthesis.

They found that BNIP-H associated with a motor protein known as kinesin. Kinesin is well known for transporting cellular cargo over large distances, for instance, from one end of a nerve cell to the other, by 'walking' along 'tracks' formed by long filamentous structures called microtubules. While BNIP-H moves along the neurites via kinesin, it also acts as a scaffold, forming a complex with ACL. Once this complex arrives at neurite terminals, ChAT is recruited for ACh synthesis and release, triggering a signaling pathway that promotes and reinforces neurite outgrowth.

Extending their studies into living organisms, the scientists found that mutation of the BNIP-H gene in zebrafish causes disrupted ACh signaling. This led to impaired neurite outgrowth, abnormal development of motor neurons, and consequently these fish exhibited severe movement defects. Interestingly, mutation of BNIP-H in humans can cause a rare genetic disorder called Cayman ataxia. This disease is characterized by inability to coordinate and control muscular movements, similar to the symptoms seen in the BNIP-H mutant zebrafish.

This study revealed how BNIP-H spatially regulates ACh to eventually promote neurite outgrowth. The discovery that Cayman ataxia could arise from dysregulation of this molecular mechanism provides new avenues for investigation and therapy. Promisingly, this mechanism may also shed light on the significant drop in ACh levels and signaling that are a hallmark of Alzheimer's disease and dementias, which could lead to novel drug targets to treat these debilitating disorders.

BNIP-H defines the precise localization, duration and strength of acetylcholine signalling that determines the growth of neurons and the coordination of body movements

ABOUT THE RESEARCHER:

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Principal Investigator at the Mechanobiology Institute, and Associate Professor at the Department of Biological Sciences, NUS. His lab focuses on cell signaling, developmental biology, and mechanobiology.

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REFERENCE:
Sun et al, BNIP-H recruits the cholinergic machinery to neurite terminals to promote acetylcholine signalling and neuritogenesis. Developmental Cell (2015), dx.doi.org/10.1016/j.devcel.2015.08.006

Molecular Mechanobiology

FORCES ACTING FAR AND WIDE

How local forces induce distant effects on actin remodeling

Written by Sruthi Jagannathan. Illustrations by Chun Xi Wong and Cindy Zhang.

For every action there is a reaction. Pierce the skin of your fingertip and the painful effects at the site of injury will be immediate. What you may not realize is that other responses are also underway far from the injury site, from within your brain to the muscles of your arm.

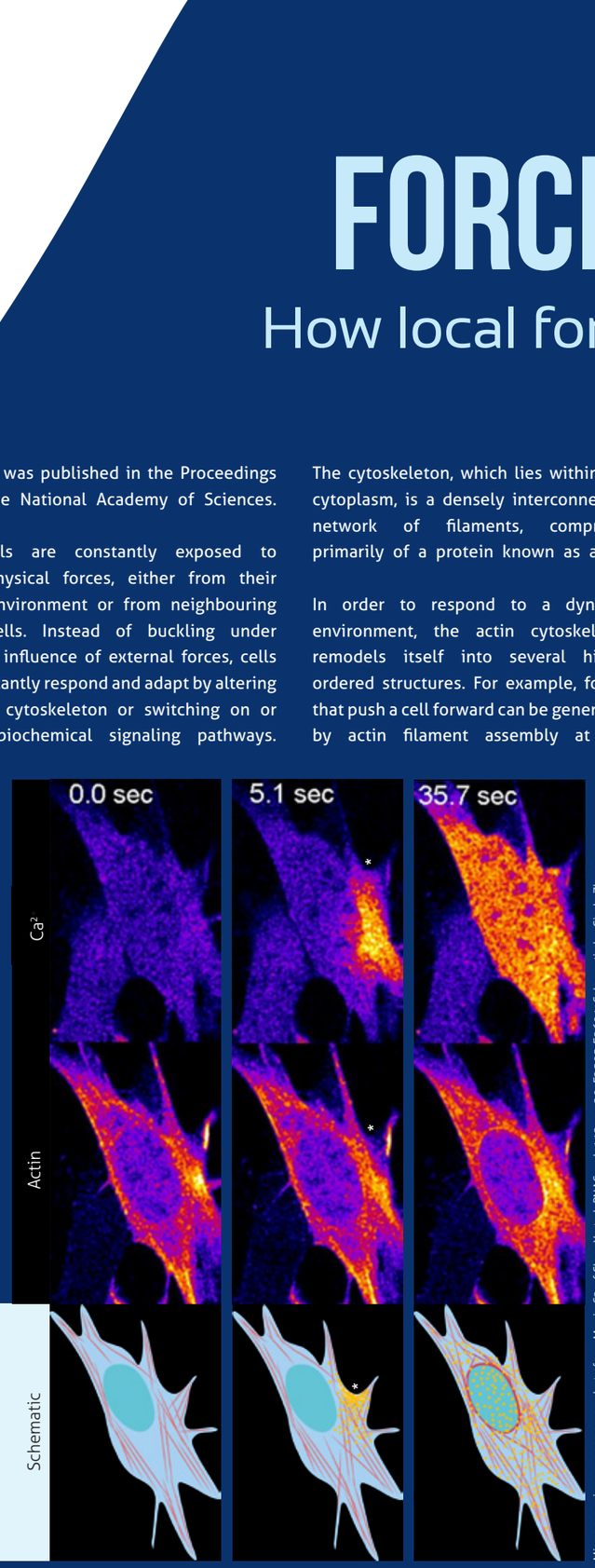
Cells, like people, can sense when forces are applied to them. While scientists had begun to understand the effects that occur within the immediate vicinity of force application, little was known about the effects that occur at cellular sites further away from where force was applied.

This question was addressed in a study led by scientists from the Mechanobiology Institute (MBI), National University of Singapore, in collaboration with scientists from the Department of Biology, New York University. Their work was published in the Proceedings of the National Academy of Sciences.

Cells are constantly exposed to physical forces, either from their environment or from neighbouring cells. Instead of buckling under the influence of external forces, cells constantly respond and adapt by altering their cytoskeleton or switching on or off biochemical signaling pathways.

The cytoskeleton, which lies within the cytoplasm, is a densely interconnected network of filaments, comprised primarily of a protein known as actin.

In order to respond to a dynamic environment, the actin cytoskeleton remodels itself into several highly ordered structures. For example, forces that push a cell forward can be generated by actin filament assembly at the front of the cell, into structures called lamellipodia. Similarly, actin filaments bundle into 'stress fibres' and line up parallel to the direction of forces. This occurs in response to stress. Previously, most studies showed that such cytoskeletal reorganization occurs at regions in close proximity to where the force is applied, however it remained unclear how far such effects actually reached.



Microscopy images are screenshots from Movie S2 of Shao X et al, PNAS, vol. 112 no. 20, E2595-E2601. Schematic by Cindy Zhang

Figure: Force-induced Ca^{2+} influx precedes perinuclear actin assembly. Time-lapse images showing fluorescence intensity of (Upper) the Ca^{2+} indicator G-CaMP and (Middle) red fluorescent protein (RFP)-Lifeact on force application. The arrow indicates the perinuclear actin rim and the * shows the point of force application.

As it was known that external forces can influence gene expression, which takes place within the nucleus, deep inside the cell, MBI researchers speculated that mechanical stimuli could reach this region primarily by actin remodeling and this was tested using an atomic force microscopy probe to apply localized forces at the cell periphery.

The researchers discovered that localized mechanical stimulation could have distant effects on actin remodeling. Described for the first time was an actin structure that formed as a ring along the outer boundaries of the nuclear envelope. This structure, which they named the actin rim, was observed to be short-lived, disappearing when the forces were removed. The formation of the actin rim was explained by a prominent increase in actin filament assembly around the nucleus. This was found to result from biochemical signaling. Specifically, a rapid release of calcium ions within the cytoplasm occurred immediately after the application of force. The calcium ions act as biochemical messengers that relay the force signals to an actin regulatory protein, inverted formin 2 (INF2). INF2, along with other mediators, functions to bring together actin proteins dispersed in the cytoplasm, leading to their assembly into filament structures.

This study enhances our understanding of how forces applied on a small spot on the cell surface can travel across the cell and elicit responses at distant regions. It was proposed that the actin rim could serve as a means to transport DNA-regulatory signals from the cytoplasm into the nucleus. Furthermore, the formation of the actin rim may act as a shield to protect DNA from forces and stabilize nuclear functions.

REFERENCE:
Shao X. et al, Mechanical stimulation induces formin-dependent assembly of a perinuclear actin rim. Proceedings of the National Academy of Sciences. 2015. 112(20):E2595-E2601. doi: 10.1073/pnas.1504837112.

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. His lab is interested in nuclear mechanics and genome regulation.

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MULTICOLOUR SUPER RESOLUTION IMAGING

A method to monitor dynamic protein binding at subsecond timescales

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

MBI researchers from the lab of Prof. Michael Sheetz developed a new method utilizing super-resolution microscopy, to determine the length of stretched proteins in living cells, and monitor the dynamic binding of proteins, at sub-second timescales. This study was published in Nano Letters.

Monitoring force-induced talin stretching and the dynamic binding of vinculin to talin

Cells are constantly exposed to mechanical forces. These signals influence cellular decision making by providing information cells need to determine how much of a particular protein to produce, when a specific gene should be expressed, or even whether a cell should move or remain where it is. Such information is crucial, for example, in maintaining the health, integrity and repair of tissues as we age. A clear example of when cells are exposed to forces is when we walk. Stretching or pulling forces are generated within our muscles, and these are passed through the muscle to connective tissue and bone. Although this information is generated at a tissue level, it converges on single cells within those tissues, and is detected and measured by subcellular, protein based machines.

To measure the forces applied to a cell, specialized proteins may be deformed. A common way that this occurs is when a protein is stretched, just like how an elastic band stretches when subjected to pulling forces. Stretching of proteins can expose regions within them that are otherwise hidden. These regions can serve as docking sites for the attachment of other proteins. This leads to a snowball effect, wherein more and more proteins are able to bind, and larger molecular complexes or machines form to mediate a specific cellular function. This phenomena was explored by MBI Director, Prof. Michael Sheetz, Senior Research Fellow Dr. Felix Margadant and PhD student Xian Hu (Edna), in work focused on characterizing the stretching of a force-sensing protein known as talin, and establishing the effect it has on the binding of another protein called vinculin.

Although several studies have shown the force-induced stretching of talin and talin-vinculin binding in vitro, simultaneous visualization of both these events and their correlation to specific cellular functions was not previously possible in living cells due to the rapid time scales at which they occur. Also, carrying out multicolour super resolution imaging in living cells is still very difficult. To overcome these challenges, Prof. Sheetz and Ms Hu developed a novel, and highly advanced super-resolution imaging method, that allowed them to simultaneously monitor talin length in living cells, as well as the dynamics of vinculin binding, at single molecule level and millisecond timescale.

By attaching different fluorescent molecules (GFP and mCherry), to each end of the talin and a third fluorophore (Atto655) to vinculin, the researchers could monitor the precise subcellular location of each protein, and confirm that when talin was being stretched, vinculin bound to newly exposed sites. Interestingly, their findings often revealed clustered binding, with 5 or more vinculin molecules binding to talin in one second. Moreover, the binding of the first few vinculins seemed to energetically favour the successive binding of more vinculin molecules. Correlating vinculin binding dynamics with the amount of talin stretching, the researchers noted that maximum vinculin binding occurred at one specific end of talin (the N-terminal region), when talin was stretched to approximately 180nm.

Understanding how talin and vinculin respond to stretching forces is crucial to understanding how cells respond to forces in our bodies. In this case, both proteins are found in larger molecular machinery called focal adhesions, which physically connect the interior of a cell with the material that is surrounding the cell, the extracellular matrix.

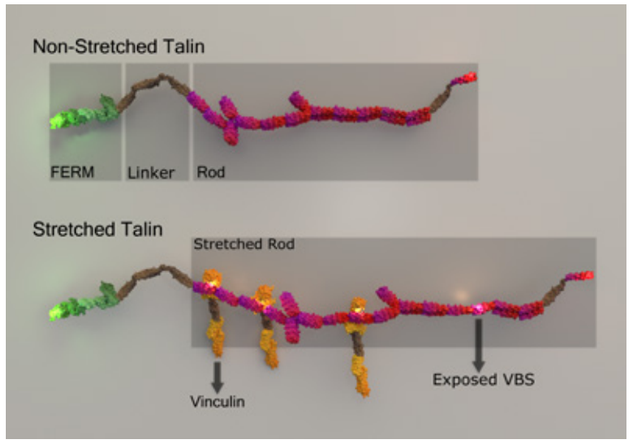
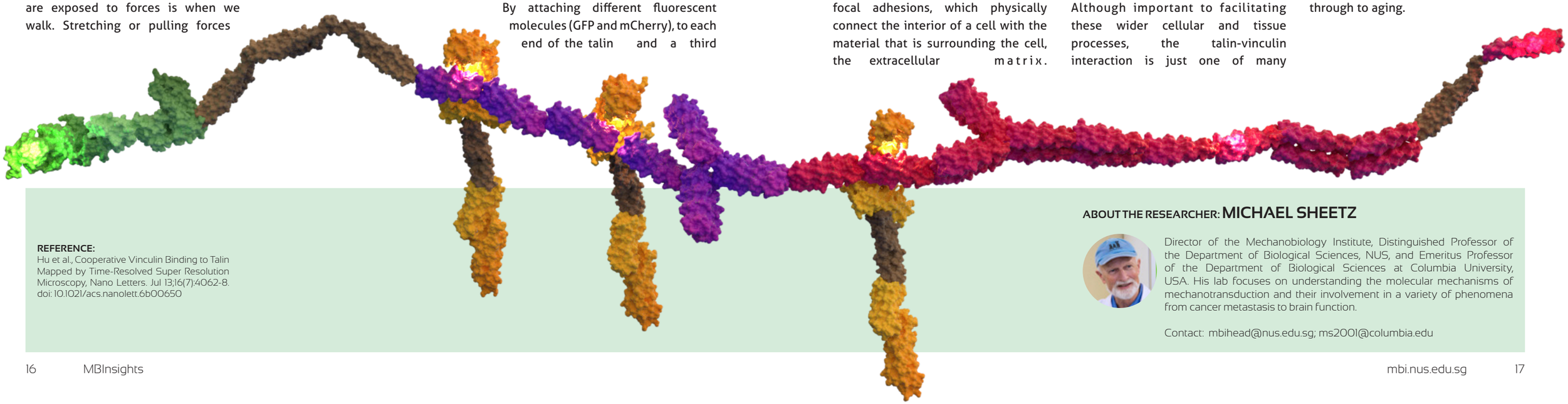


Figure: Talin stretching and stretch-induced vinculin binding.

Focal adhesions primarily function as signal relaying centres, and the information they transfer can induce cell growth and cell movement. When this signal processing is disrupted, or is not regulated, disease states arise and the body's ability to heal wounds, or maintain tissue integrity as we age becomes impaired.

Although important to facilitating these wider cellular and tissue processes, the talin-vinculin interaction is just one of many protein interactions to respond to force. It is hoped that this newly described method will pave the way for researchers to dissect other protein interactions, both within focal adhesions, and in other molecular machines, to improve our understanding of the many force-driven cellular processes that arise during development and continue through to aging.



REFERENCE:
Hu et al., Cooperative Vinculin Binding to Talin Mapped by Time-Resolved Super Resolution Microscopy, Nano Letters. Jul 13;16(7):4062-8. doi: 10.1021/acs.nanolett.6b00650

ABOUT THE RESEARCHER: MICHAEL SHEETZ

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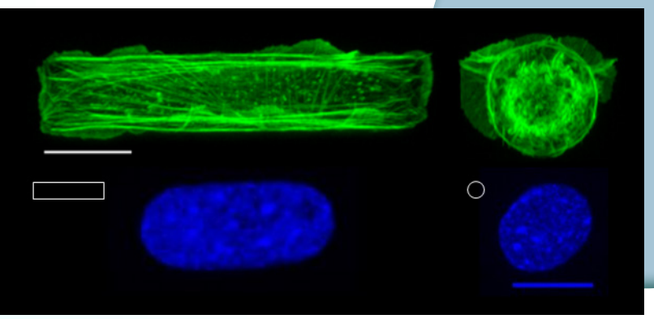
Molecular Mechanobiology

SHAPING CELL BEHAVIOUR

Clues to how external forces control nuclear activity

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

The nucleus is often thought of as a rigid, spherical structure that is solely responsible for storing the cell's genetic material; DNA. Such a notion stems from early observations of cells fixed to microscopy slides. However, advances in the imaging of living cells have revealed that the nucleus is in fact a highly dynamic structure, and one that can adopt different shapes and sizes. What geometry it adopts will depend on the shape of the cell, and the environment in which the cell is growing. This characteristic is called nuclear plasticity or deformability.



Importantly, the shape and size that a nucleus adopts has a profound impact on the life of the cell, well beyond how it appears under a microscope. It will, in fact, affect how the DNA is stored, how it is unpacked, and which genes are decoded and when.

A question that has remained unanswered however, is how forces from outside the cell regulate the dynamics of the nucleus, and hence, the DNA housed within. Clues that answer these questions began to emerge through the work of a research team from the lab of G.V. Shivashankar, Deputy Director at the Mechanobiology Institute (MBI), National University of Singapore. These findings were published in the Proceedings of the National Academy of Sciences.

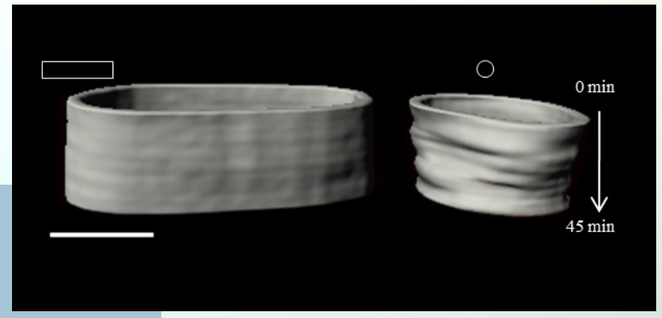
REFERENCE:
Makhija E, Jokhun DS, Shivashankar GV, Nuclear deformability and telomere dynamics are regulated by cell geometric constraints. Proc Natl Acad Sci U S A. 2016 Jan 5; 113(1):E32-40. doi: 10.1073/pnas.1513189113

Images taken from Fig. 1a, 1b, 1c, and Fig S8.

How external forces regulate the nucleus

Nuclear deformability is greatly determined by the cell's physical environment. For example, the stiffness of the substrate on which the cell grows, and the specific geometry the cell adopts. This is important because in the body, cells grow in various conditions, and on many different surface types. Different cell types will also produce different proteins at different times.

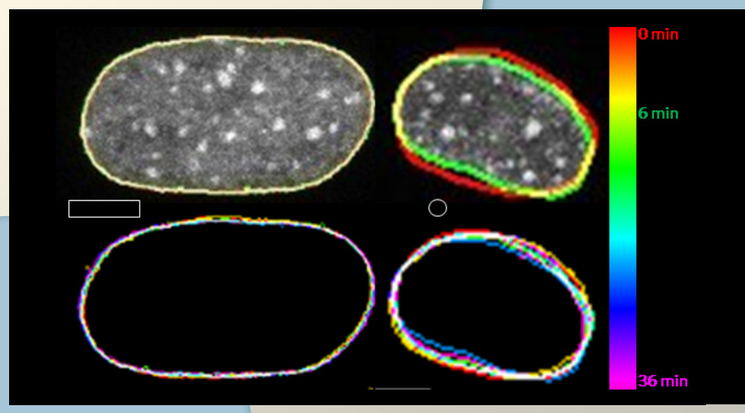
The underlying factor that allows cells to transmit information about the environment, is the mechanical signal generated as the structural framework of proteins inside the cell, called the cytoskeleton, organizes itself with respect to the cell geometry. This mechanical signal is transmitted into the nucleus through a physical link between the cytoskeleton, and a similar structural protein framework within the nucleus, called the nucleoskeleton. How these mechanical signals regulate nuclear dynamics was the major question addressed by Makhija et al. By artificially creating two extreme cell geometries, circular and rectangular, for the same type of cell, the group showed that different cell geometries result in different cytoskeletal organization. This in turn creates different mechanical signals, nuclear deformability and dynamics. They observed that a nucleus in a circular cell is more deformable and dynamic than the nucleus in a rectangular cell.



The group also monitored the activity of specific regions of chromatin, or packaged DNA, that are not normally decoded, called heterochromatin, as well as regions at the ends of chromosomes, which are called telomeres. They found that each of these regions are more dynamic in cells with a circular geometry.

This raised the question as to why a circular geometry produces a more deformable nucleus, and more active DNA in regions that are normally less active. What was uncovered was the presence of minute cytoskeletal structures, made up of the proteins actin, which is the building block of the cytoskeleton; myosin, which makes the cytoskeleton contract; and formin, which promotes rapid elongation of the cytoskeletal filaments. The nucleoskeleton of these circular, deformable nuclei was also found to lack the nucleoskeleton protein, lamin A/C.

The importance of these findings is far reaching. As cells in the body grow in different locations, they adopt different shapes and sizes. Stem cells, which have yet to adopt a specialized function, generally have a highly deformable nucleus, and are associated with more active DNA processing. This allows the DNA to be decoded more frequently, and produce a wider range of proteins that dictate cell behaviour and allow the cell to adopt a specialized function. On the contrary, cells that have already become specialized, require less active DNA, and are thus less deformable.



This work suggested for the first time how mechanical changes brought about by the environment in which a cell is growing, influences the integrity of the genome, as well as the decoding of genetic material to define cell behaviour.

ABOUT THE RESEARCHER:
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Molecular Mechanobiology

SALMONELLA LIFESTYLE CHOICES

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

A study led by Senior Research Fellow Dr. Stuti Desai, and Prof. Linda Kenney, discovered that the bacterial protein SsrB is the molecular switch for determining whether *Salmonella* infections become acute and virulent, or remain in a dormant carrier state. This study was published in eLife.

A virulent or dormant lifestyle?

Salmonella is responsible for many human gastrointestinal diseases, ranging in severity from diarrhoea to typhoid fever. Once it has entered the host body, *Salmonella* is able to successfully thrive by adopting one of two distinct lifestyles.

The first of these is the virulent lifestyle, where *Salmonella* resides in a modified vacuole inside the macrophage after engulfment. Within this *Salmonella*-containing vacuole (SCV), the bacteria are able to survive, reproduce, and activate virulence factors, and eventually escape the macrophage to spread infection. In the dormant lifestyle, multiple *Salmonella* join together to form a biofilm, a matrix-encased bacterial layer which can adhere to epithelial cells, gallstones, and tumours. In this communal state, *Salmonella* are highly resistant to attack from antimicrobials or the host immune system, and remain persistent as well as asymptomatic in the body until conditions favour switching to the virulent lifestyle.

These two lifestyles are controlled by two-component regulatory (TCR) systems, which consist of a membrane-bound sensor protein and a response regulator protein inside the bacteria. Much like putting your hand out of a window to check the weather, the sensor protein enables the bacteria to probe the environment. After measuring different factors such as acidity, antibiotic levels, or osmolarity, the sensor protein sends a chemical signal via transfer of a phosphate group to the response regulator protein. The phosphorylated response regulator is now activated, and it can change the expression of target genes, which drive the bacterial response to the external stimuli.

A classic example of a TCR system is SsrA/SsrB, which is essential for establishing the virulent lifestyle. When the SsrA sensor detects that *Salmonella* is in the acidic SCV, it signals the SsrB response regulator. Phosphorylated SsrB activates virulence gene expression and synthesis of disease-causing needle and effector proteins.

Despite the identification of the gene *csgD* as the master regulator of biofilm formation, the mechanism by which *Salmonella* switches between the two lifestyles was still unknown. By elegantly combining molecular biology and biochemical techniques with confocal and scanning electron microscopy, a multidisciplinary team of scientists led by Senior Research Fellow Dr. Stuti Desai and MBI Principal Investigator Prof. Linda Kenney discovered that SsrB plays a dual role in regulating the dormant and virulent lifestyle. The first hints for this came from *Salmonella* lacking SsrB, which were unable to form biofilms. However, unlike virulence gene activation, SsrB did not need to be phosphorylated by SsrA before regulating biofilm formation.

Histone-like nucleoid-structuring protein (H-NS) silences many *Salmonella* genes, including *csgD*. This gene silencing takes places through H-NS binding to DNA, in order to form a stiff nucleoprotein filament that physically blocks DNA reading enzymes from accessing the gene. In collaboration with MBI PI Assoc. Prof. Yan Jie and Dr. Ricksen Winardhi's expertise in single molecule manipulation, the researchers applied atomic force microscopy to observe *Salmonella* DNA at the nanoscale, which revealed that unphosphorylated SsrB binds to DNA and causes it to bend at an angle of around 82 degrees. This bending relieves H-NS induced stiffening and silencing of *csgD*, allowing its expression and promoting formation of biofilms.

This study revealed that SsrB is the key decision maker for determining *Salmonella* lifestyle choice. In conditions that suit infection, the SsrA/SsrB TCR system phosphorylates SsrB, which then activates genes driving the virulent lifestyle.

However, in multicellularity-promoting conditions, the presumed lack of activity from SsrA leaves SsrB unphosphorylated, and its anti-silencing of *csgD* leads to the bacteria assembling into biofilms to adopt the dormant lifestyle. Understanding the dual role of SsrB may lead to new antibiotic strategies targeted against the different lifestyles of *Salmonella*.

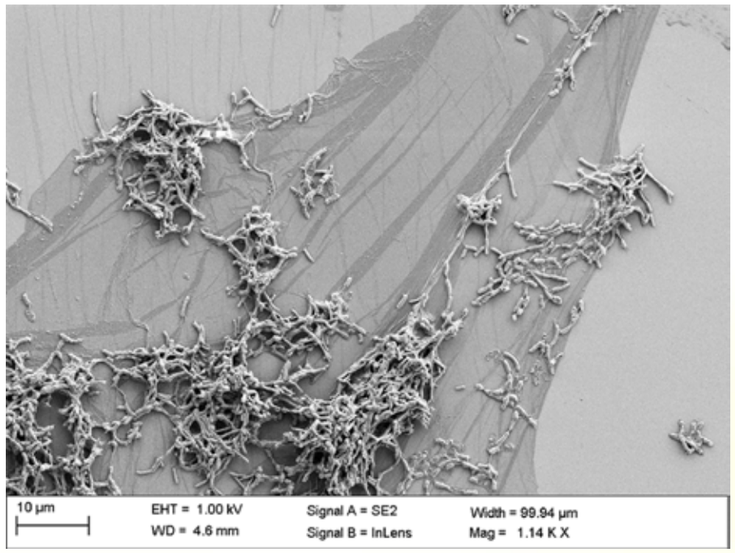

Figure: SEM image of a *Salmonella* biofilm. Image provided by Dr. Stuti K Desai.

ABOUT THE RESEARCHER:

Stuti K Desai

Senior Research Fellow in the lab of Prof. Linda Kenney at the Mechanobiology Institute.

REFERENCE:
Desai et al., The horizontally-acquired response regulator SsrB drives a Salmonella lifestyle switch by relieving biofilm silencing, February 2, 2016, eLife 2016; 5: e10747, doi: 10.7554/eLife.10747

H-NS PROTEINS AS GENE SILENCERS

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

Deoxyribonucleic acid or DNA, one of the major macromolecules found in cells, is considered the blueprint of life. It contains all the information required to carry out cellular activities and sustain life in every living organism, from the simplest bacteria to highly evolved humans.

In bacteria, DNA is organized into a structure called a nucleoid, which is accomplished by the action of nearly a dozen of abundant DNA-binding proteins known as nucleoid-associated proteins (NAPs). NAPs also dynamically influence gene transcription globally. Thus, NAPs play two important roles: nucleoid organization and transcription regulation. A better understanding of the DNA-binding properties of NAPs will provide crucial insights into the mechanisms underlying the function of these bi-functional proteins.

H-NS binds DNA and forms gene-silencing nucleoprotein filaments

The H-NS family of NAPs belongs to a set of proteins widespread in gram-negative bacteria, executing a special function as global gene silencers and playing critical roles in bacterial pathogenesis. A research team from the MBI, comprised of Principal Investigators Jie Yan and Linda Kenney, and several team members including post-doctoral fellow Ricksen Winardhi, has been working together for several years to understand the relation between DNA binding and the gene-silencing mechanisms of H-NS proteins. Based on accurate measurements using advanced single-molecule manipulation and imaging methods, this collaboration has led to a discovery that a rigid nucleoprotein filament formed by H-NS proteins on double-stranded DNA provides the basis for their gene silencing activity.

The discovery of H-NS nucleoprotein filaments supplies a structural basis for understanding its gene silencing function. It also enables an understanding of how H-NS mediated gene silencing can be relieved by a set of anti-silencing proteins to activate transcription. These findings have led to more than ten publications that have made a significant impact on the field. An invited Biophysical Perspective published in the Biophysical Journal integrated these findings, and highlighted the role of H-NS proteins in bacterial nucleoid compaction and the implications for its gene-silencing functions.

ABOUT THE RESEARCHERS:

JIE YAN

Principal Investigator at the Mechanobiology Institute, Centre of Bioimaging Sciences, and the Dean's Chair Associate Professor at the Department of Physics, NUS. His lab uses advanced single-molecule manipulation and imaging to investigate mechanosensing and DNA.

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LINDA J KENNEY

Principal Investigator at the Mechanobiology Institute, NUS, and Professor of Microbiology and Immunology and Adjunct Professor of Bioengineering at the University of Illinois-Chicago. Her lab focuses on signal transduction and the regulation of gene expression in prokaryotes.

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REFERENCE:

Winardhi RS et al. H-NS regulates gene expression and compacts the nucleoid: Insights from single molecule experiments. Biophysical Journal. 2015. 109 (7). 1321-1329. doi: 10.1016/j.bpj.2015.08.016

DEFINING ADHESION CLUSTERS

Written by Lakshmi Ramachandran. Illustration by Diego Pitta de Araujo.

Research from the labs of Pakorn Kanchanawong and Ronen Zaidel-Bar discovered the molecular mechanisms responsible for the formation of the adherens junction at the nanoscale level. This research was published in Developmental Cell.

How are cell-cell adhesions initiated?

Although the cells that make up our body are functional units by themselves, they need to interact with each other and their environment to fulfill all their functions. Cells stick to one another as well as to their substrate through physical contacts called cell adhesions. Apart from serving as physical connections that enable cells to form tissues, cell adhesions also allow the cells to sense, signal, and respond to physical or chemical changes in the environment, as well as interact with neighbouring cells.

This is due to the structure of adhesion sites, or cell-cell junctions, which extend through the cell surface into the cell's interior. At cell-cell junctions, adhesion receptors at the cell surface are linked via adaptor proteins to the cytoskeleton, a structural scaffold inside the cell composed of filamentous proteins like actin. Epithelial cadherin (E-cadherin) is a major adhesion receptor protein which forms a prominent cell-cell adhesion complex called the adherens junction.

Traditionally it was thought that clusters of E-cadherin merge to form a thick belt along the cell membrane between adjacent cells. The binding of individual E-cadherin proteins was thought to drive adhesion, with clusters formed in an adhesion dependent manner, before merging and becoming uniformly distributed over time. This has long been the prevalent notion, based on conventional microscopy, which is limited in its ability to clearly visualize structures as small as the adherens junction or E-cadherin cluster.

However, the findings in this study disprove this notion. Using a combination of an advanced imaging technique called super-resolution microscopy along with quantitative analysis and mutational studies, MBI Principal Investigators Asst. Profs. Ronen Zaidel-Bar and Pakorn Kanchanawong, and graduate student Yao Wu showed that cell-cell adhesions are initiated by small clusters of about five E-cadherin molecules.

Super-resolution imaging allowed the nanoscale architecture of the adherens junction to be observed, and distinct, evenly sized E-cadherin clusters were monitored both in the incipient and mature cell-cell adhesions. The precursor E-cadherin cluster forms independently of adhesion, even when mutations prevent E-cadherin interactions, indicating that their formation relies on an alternative mechanism.

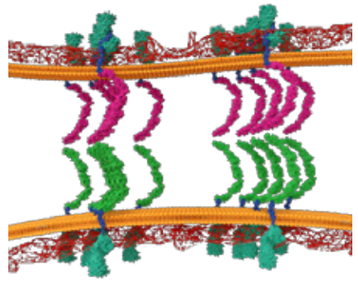

Figure: The formation of adherens junctions are initiated by small clusters of approximately five E-cadherin molecules.

As more E-cadherin molecules were recruited from neighbouring cells, the clusters became denser especially at their core. However, E-cadherin clusters never increased in size or merged to form the hypothesized belt. Instead, the actin cytoskeleton was seen to fence E-cadherin clusters, thereby preventing them from merging.

These newly identified steps of adherens junction assembly, organization and maintenance advance our understanding of how adherens junctions adapt to dynamic changes in the behaviour of epithelial cells. Regulating essential functions such as cell shape, movement and rearrangement is vital for maintaining epithelium integrity, and is also important for tissue repair in wound healing and disease.

ABOUT THE RESEARCHERS:

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RONEN ZAIDEL-BAR

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

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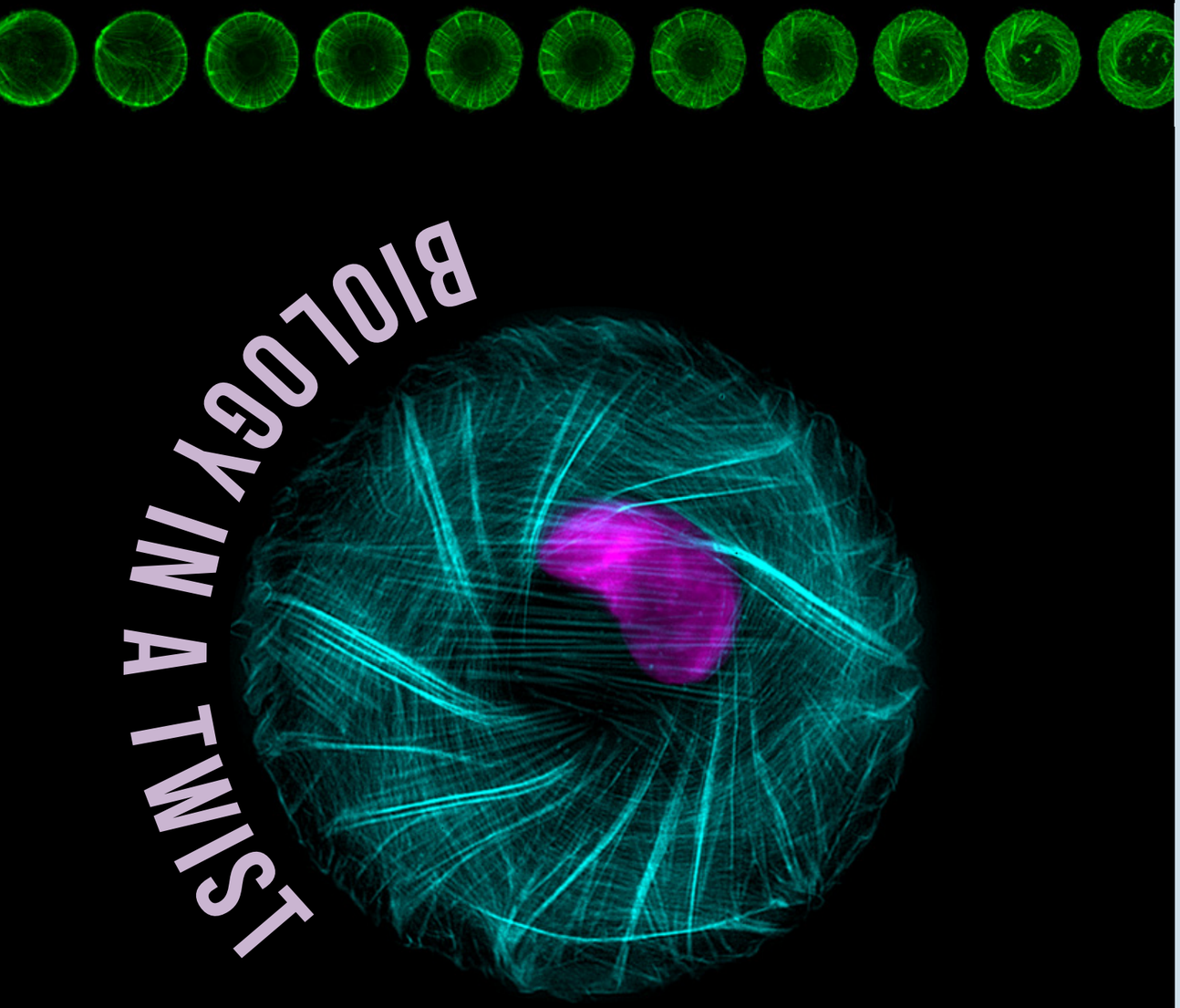
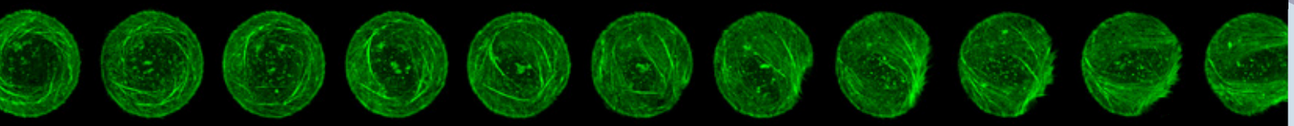
REFERENCE:

Wu Y, et al. Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions. Developmental Cell. 2015. 32(2): 139-154. doi.org/10.1016/j.devcel.2014.12.003



DECIPHERING THE ORIGINS OF CELL BEHAVIOUR

Written by Steven Wolf.



Researchers from the laboratory of Prof. Alexander Bershadsky have made significant advances in our understanding of cellular biology; providing evidence that the inherent 'handedness' of molecular structures directs the behaviour of individual cells and confers them the ability to sense the difference between left and right. This study was published in Nature Cell Biology.

Cellular decision making

Our bodies are made up of hundreds of different types of cells, each of which performs a unique and highly specialized task. Traditionally, the ability of cells to specialize in a given function was attributed to its genetic code. However, it is becoming increasingly clear that cells do not simply live by a set of inherited or pre-determined instructions. Instead, 'cellular decisions' are made dynamically, much like humans make decisions based on the information provided to us by our senses.

Although cells do not have the ability to see or hear, they do possess sensory structures that allow them to detect and measure various environmental stimuli. The application of mechanical force to the cell, for example, will be felt and the cell will respond accordingly. One of the most prominent cellular responses is to change shape and this property is reflected in the varying shapes of specialized cells.

Cellular senses have been attributed to various force-sensing cellular structures such as the cytoskeleton. This structure differs significantly from its namesake, the human skeleton, by being highly dynamic and playing roles in addition to the provision of structural support. For example, this network of molecular filaments or cables also generates internal forces that drive shape changes and even motility. As the cytoskeleton develops, individual protein filaments grow and shrink. They bundle together to form thicker fibres, and they move or contract. Each of these processes is collectively known as 'cytoskeleton dynamics'.

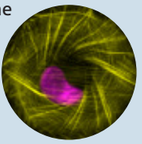
The question that has long intrigued scientists is how cytoskeleton dynamics can direct the behaviour of different cell types. To investigate this, MBI researchers Prof. Alexander Bershadsky and Dr. Yee Han Tee, in collaboration with researchers from the USA and Israel, observed the cytoskeleton in cells that were confined to a small circular area, using a technique known as "micro-patterning". This prevented the cells from changing shape and thus provided the researchers an unhindered view of cytoskeleton dynamics.

Determining chirality

What was detected came as a surprise to the researchers. A pronounced left-right asymmetry was observed during cytoskeletal organization. This asymmetry, which appeared as a whirlpool, with filaments moving anticlockwise inside the cell, was found to originate from the inherent twist that is present in individual actin filaments. This helical twist occurs naturally as individual actin proteins join together to form the long actin cables that make up overall structure. This seemingly simple property has profound consequences as it suggests that the asymmetry of a single protein is translated to the asymmetric behaviour of a whole cell. This is akin to the twist of a screw or bolt directing the function or behaviour of the machine in which it is placed.

The ability of cells to distinguish between left and right is a phenomenon that continues to fascinate scientists. It is clear from this study that the asymmetry inherent in molecular structures can define the behaviour of whole cells, and this provides new insight into the ability of cells to 'make decisions' based on the mechanical properties of its environment.

However, these findings also raise fascinating questions as to whether the same phenomenon can influence the formation and function of our organs, or even affect organism behaviour. Indeed relatively simple biological systems, such as cells grown on defined patterns, display a pronounced asymmetry in their movement. At the other extreme, brain function and human cognition is dependent on the asymmetric behaviour of nerve cells. The possibility that the inherent asymmetry of molecular structures can define cell, tissue or even organism behaviour will undoubtedly drive further studies for years to come.



Cartoon schematic showing how chirality is established through the anticlockwise organisation of actin filaments

ABOUT THE RESEARCHER:

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REFERENCE
Tee YH. et al, Cellular chirality arising from the self-organization of the actin cytoskeleton. Nature Cell Biology; doi: 10.1038/ncb3137

SURVIVING AN IMMUNE ATTACK

HOW DOES *SALMONELLA* THRIVE IN THE ACIDIC MACROPHAGE?

Written by Andrew Wong. Illustrations by Diego Pitta de Araujo and Cindy Zhang.

Salmonella-related diseases have a huge impact worldwide, both in first and third-world countries. Despite improvements in hygiene and availability of antibiotic drugs and vaccines, every year around 100 million people worldwide are likely to suffer from food poisoning or typhoid fever. Understanding how *Salmonella* is able to bypass our immune system and cause infection is a key global health need.

A team led by Prof. Linda Kenney has discovered that *Salmonella* acidifies itself to camouflage and protect against acid digestion from the immune system, and actually uses these low pH conditions to switch on essential genes to continue spreading infection throughout the body. This work was published in PLoS Biology.

Salmonella self-acidifies to spread infection

Suffering from food poisoning is an unfortunate experience that most of us are likely to encounter during our lifetimes. One of the most common causes of food poisoning is bacterial infection from *Salmonella*, typically by ingestion of contaminated food or water. After passing through the digestive system, *Salmonella* are recognized by macrophages, the sentinel cells of the immune system. These cells are highly specialized to identify and remove harmful substances, cellular debris, and bacteria.

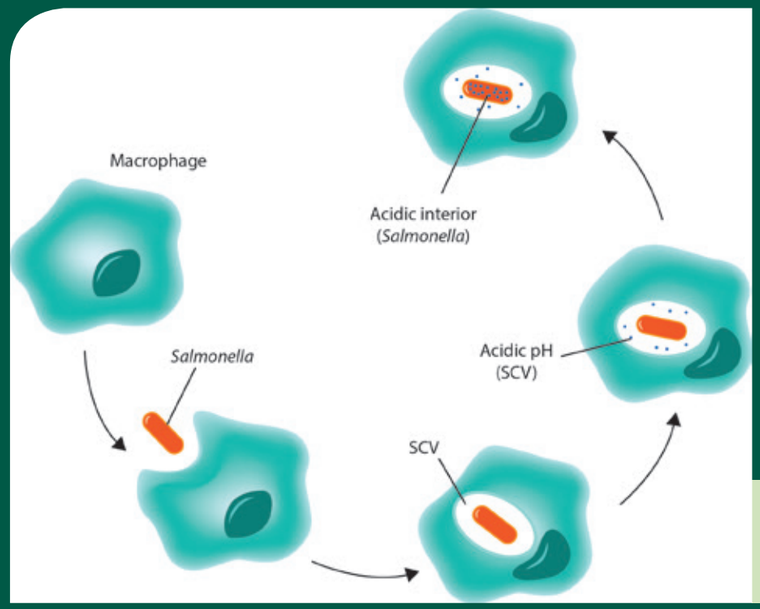
Macrophages surround and engulf *Salmonella*. The macrophage then contains the bacteria within an intracellular compartment, called the *Salmonella*-containing vacuole (SCV), and lowers pH levels, effectively becoming an acid bath designed to dissolve and kill the bacteria.

However, *Salmonella* has evolved over the years to survive, and even reproduce in the SCV. Although the existing viewpoint is that *Salmonella* somehow manages to counteract the acidic surroundings and maintain neutral conditions, it is still unclear how *Salmonella* is able to survive after being engulfed by macrophage. Using the I-switch biosensor, Drs. Smarajit Chakraborty and Hideaki Mizusaki from the lab of Prof. Linda Kenney at MBI, revealed that *Salmonella* does not neutralize the acidic environment, but instead it acidifies itself to match its surroundings.

The I-switch is a flexible, double-strand of DNA which has different fluorescent molecules attached to each end. At low pH (acidic conditions), the DNA acts like a hinge, bringing the two ends together, resulting in activation of the fluorophores or FRET. After inserting the I-switch into *Salmonella*, the researchers were able to measure the acidity of the bacterial interior (cytoplasm), in real-time. They discovered that after *Salmonella* has been engulfed in the acidic SCV, the pH of the cytoplasm rapidly drops. Within minutes, *Salmonella* becomes approximately 150 times more acidic, acclimatizing to the external conditions of the SCV.

Remarkably, the authors also found out that this self-acidification of the bacterial cytoplasm was an essential step for activation of virulence genes and proteins. Once switched-on, these virulence factors allow *Salmonella* to survive and reproduce in the SCV, and the bacteria are eventually secreted from the macrophage to further the spread of infection throughout the body. This study redefines our understanding of how *Salmonella* survives after being targeted for killing by our immune system.

Previously, it was thought that *Salmonella* must first neutralize the acidic environment of the SCV in order to activate virulence factors. Now, for the first time, MBI scientists revealed that *Salmonella* has actually adapted to sense, respond and even thrive in these anti-bacterial conditions. With this new knowledge of how *Salmonella* has evolved to embrace acidic attack by the immune system in order to spread disease, it may be possible to design new treatments and antibiotic drugs to combat the immense health and economic burden from *Salmonella*-related diseases.



Schematic depiction of how *Salmonella* acidifies itself to survive the acidic environment following engulfment by macrophages.

ABOUT THE RESEARCHER: LINDA J KENNEY
Principal Investigator at the Mechanobiology Institute, Singapore, and Professor of Microbiology and Immunology at the University of Illinois-Chicago. Her lab focuses on signal transduction and the regulation of gene expression in prokaryotes.

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REFERENCE:
Chakraborty S, Mizusaki H, and Kenney LJ. A FRET-Based DNA Biosensor Tracks OmpR-Dependent Acidification of Salmonella during Macrophage Infection. PLoS Biol. 2015 Apr 14;13(4):e1002116. doi: 10.1371/journal.pbio.1002116.

Cellular Mechanotransduction

ACTIVE JUNCTION FORMATION

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

An international team of researchers, led by Senior Research Fellow Kabir Biswas and Prof. Jay Groves, discovered that the formation of connections between cells is dynamically driven by active extension and retraction of membrane protrusions, and regulated by the mobility of cell adhesion molecules. This study was published in Proceedings of the National Academy of Sciences.

Is adherens junction formation an active or passive process?

Multi-cellular life depends on the ability of cells to form connections with each other. These connections are formed through interactions between a variety of proteins localized on the cell membrane of two apposing (side-by-side) cells. Once assembled, these connections enable cells to communicate through physical and chemical signals, and this cellular communication allows specialization of function and formation of dedicated organs, eventually leading to the development of complex multicellular organisms.

One of the key connections formed amongst epithelial cells is the adherens junction. It is formed by interactions between the extracellular domains of the membrane localized cell adhesion molecule, epithelial (E)-cadherin, from apposing cells. Although mature, fully-formed adherens junctions have been studied extensively, the steps involved in the formation of these E-cadherin mediated junctions continue to be unclear. A major question which still remains unanswered is whether adherens junctions form simply when a cell touches another cell, or if an active mechanism is responsible?

Previous attempts to answer questions related to adherens junction formation have used solid surfaces coated (functionalized) with cadherin. By growing live cells on these functionalized surfaces, junction formation can be reconstituted and observed in the lab. Despite advanced imaging and force measurement technology that allows scientists to examine the junction in detail, these studies have been inconclusive with respect to the mechanism of adherens junction assembly due to the immobilization of the cadherin molecules, which fails to replicate the natural dynamic mobility of cadherin on a fluid cell membrane.

In order to tackle this problem, Senior Research Fellow Dr. Kabir Biswas and Prof. Jay Groves from MBI led a team of researchers from Singapore, USA, and the UK who used artificial membranes (known as supported lipid bilayers) to accurately mimic the natural environment.

These membranes can be functionalized with protein molecules, and the density and fluidity of these molecules can be controlled to match that of a living cell. By growing cells on cadherin functionalized supported lipid bilayers, they discovered that adherens junctions form via an active, all-or-nothing, process.

Here, cells actively extend and retract thin protrusions from the membrane called filopodia. Cadherin molecules on these filopodia bind to cadherin present on supported lipid bilayers, forming an intermediate cadherin-cadherin bond. As bound cadherin is drawn back into the cell by the retracting filopodia, cadherin molecules are compressed and concentrated in that region, leading to clustering of E-cadherin and adherens junction formation.

Reduced E-cadherin mobility is required for active assembly of adherens junctions

On highly fluid bilayers cadherin is freely moving around due to fast diffusion. When the filopodia retract, the mobile cadherin molecules dissipate before compression and concentration can take place. This results in failed clustering and means a very low percentage of cells form adherens junctions on these fluid bilayers.

However, in living cells the mobility of E-cadherin is reduced as it is confined to specific membrane regions. When the natural molecular mobility of E-cadherin was reproduced by changing bilayer composition, filopodia retraction drew in enough free cadherin to pass a certain threshold, strengthening the bond and initiating E-cadherin junction formation. Once initiated, formation of the junction always continues to completion, highlighting the all-or-nothing nature.

This study further demonstrated that E-cadherin adherens junction formation is an active process, rather than a passive one. This suggested that adherens junction formation in epithelial cells is a carefully regulated process, with checks and balances present at the cellular level. Understanding how these regulatory steps influence cell adhesion will provide fresh insight on the development and maintenance of multi-cellular organisms and tissues. Problems in the regulation of adherens junction formation may also play a role in the disrupted cell-cell adhesion that is a hallmark of various human diseases, including cancer.

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REFERENCE:
Biswas KH et al. E-cadherin junction formation involves an active kinetic nucleation process Proc Natl Acad Sci U S A. 2015, doi:10.1073/pnas.1513775112

A MECHANICAL STOP SIGN

Cancer cells bypass mechanically induced dormancy

Written by Lakshmi Ramachandran. Illustration by Chun Xi Wong.

A collaborative, interdisciplinary study between two research groups at the Mechanobiology Institute, NUS, discovered the differential effects of extracellular matrix (ECM) topography on the proliferation of normal and cancerous cells. This work was published in Scientific Reports.

The environment we live in has a deep impact on our lives. For example, changes in weather, region, or people can influence our moods and determine our activities. Similarly, at a microscopic level, changes in the nature of the environment around cells – the smallest functional units that make up our body, can determine whether a cell should divide, specialize or move.

The microenvironment of the cells is primarily the ECM, which is an extremely complex substance basically composed of water, proteins and polysaccharides. The ECM chemical composition, its mechanical stiffness, as well as topographical features like furrows, ridges or pores vary in different cells and tissues. It also changes with age, during wounding, and in cancer. Researchers have long sought to understand how such physical changes in the ECM correlate with cell behaviour. However, previous studies have largely looked at how the composition and stiffness of the ECM relates to cell behaviour, meaning that very little is known about the relationship between ECM topography and cell behaviour.

In breast cancer, ECM topography changes during tumour progression as fibrous proteins like collagen organize and align, in contrast to their random organization under normal conditions. The aligned fibres enable the movement of cancer cells, fueling their ability to spread within the body. As cancer cells also possess limitless potential to divide and multiply uncontrollably, Parthiv Kant Chaudhuri, a joint graduate student from the labs of Prof. Chwee Teck Lim and Assoc. Prof. Boon Chuan Low at MBI, questioned whether such changes in ECM topography during cancer progression may also influence the proliferative potential of cancer cells.

The effect of topography on cell proliferation

To address this question, the researchers artificially created two distinct types of ECM topographies; 'micropillars', mimicking the random organization of fibrous proteins observed under normal conditions and 'microgratings', resembling the aligned organization of collagen fibres as seen during breast cancer progression. Upon observing the proliferation of normal and cancer cells on these two topographies, they discovered that microgratings, which mimicked the cancer microenvironment, restricted the proliferation of normal cells , but not cancer cells. However this restriction in the proliferation of normal cells was not seen on micropillars, which provided an environment where both normal and cancer cells proliferated.

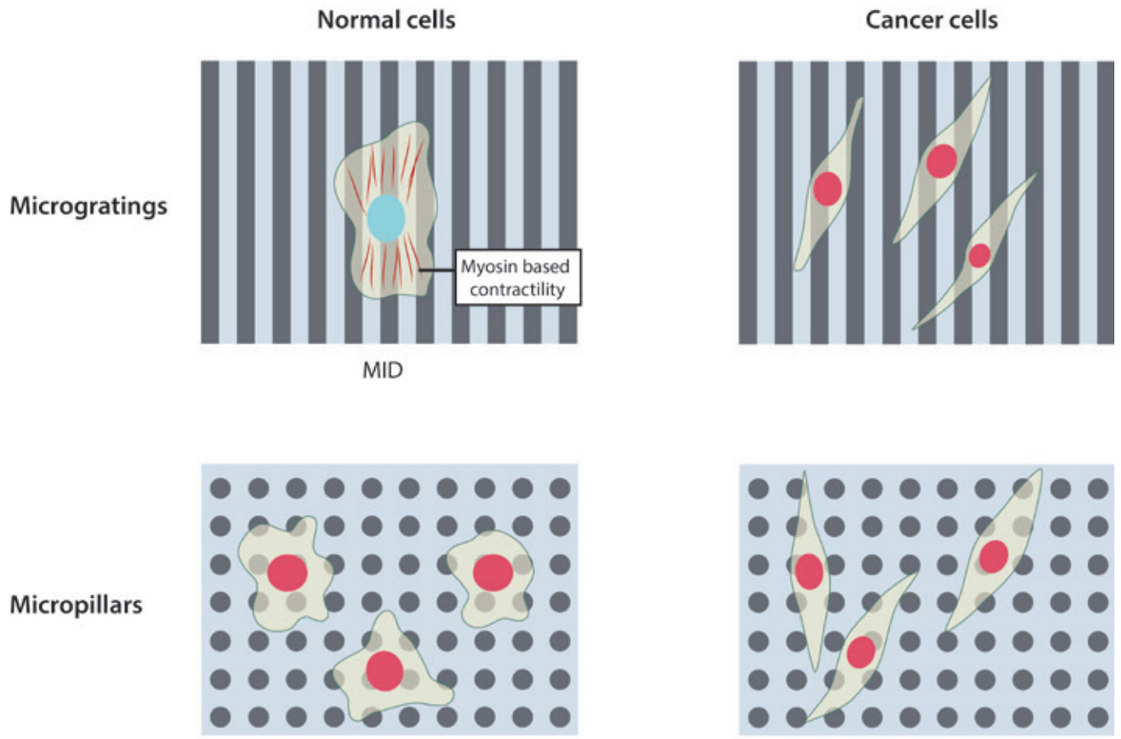

Figure: Normal cells do not proliferate (blue nucleus) on microgratings due to Mechanically Induced Dormancy (MID). Cancer cells on the other hand, bypass MID and proliferate (red nucleus). On micropillars, both normal cells as well as cancer cells proliferate.

Further investigation into what was causing the temporary dormancy of normal cells on microgratings led the research team to probe the role of the cell cytoskeleton, a dynamic and extensive network of filamentous proteins within cells that receives and transmits mechanical signals from the environment. The researchers discovered that on microgratings the cytoskeleton of normal cells is highly contractile, owing to the activity of a protein called 'myosin', which generates a mechanical signal that restricts their proliferation. They named this phenomenon 'Mechanically-Induced Dormancy (MID)'.

This study revealed a novel mechanism by which normal cells sense external topographic cues and restricts their proliferation in an environment that promotes tumour growth and spreading. This finding provides an understanding of how the cell's microenvironment plays a major role in maintaining normal tissue homeostasis during healthy conditions. Determining how tumour cells bypass this mechanism of MID may be paramount in the development of novel anti-cancer strategies that target cellular mechanisms altered by physical or mechanical cues during cancer progression.

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REFERENCE:
Chaudhuri PK et al., Topography induces differential sensitivity on cancer cell proliferation via Rho-ROCK-Myosin contractility, Sci Rep. 2016 Jan 22;6: 19672. doi: 10.1038/srep19672

WHEN STRESSED CELLS RELAX

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

An international study between Dr. Nils Gauthier, former Senior Research Fellow at the MBI, and scientists from Spain and India, showed how the cell membrane is rapidly remodelled in response to physical stress through a purely mechanical process. This work was published in Nature Communications.

Membrane remodeling via a mechanical process

Cells need to expand, stretch or move during critical physiological processes like development, breathing or wound healing. While doing so, cells frequently change their shape or size and such changes must be accommodated by the membrane that enwraps the cell. However, cell membranes are stiff, inelastic structures, and therefore the mechanism by which they accommodate these changes remains unclear.

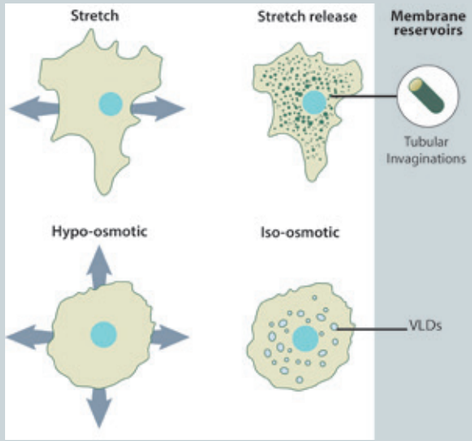
Membrane area can be increased or decreased to accommodate cell shape changes by processes that allow cargo such as proteins and macromolecules to enter or leave the cell via the cell membrane. When small areas of membrane engulf the cargo like a bubble and enter the cell, the membrane area is reduced. When similar vesicles merge with the membrane to release cargo, the membrane area is increased. Known as endocytosis and exocytosis respectively, these are active, energy dependent processes that are supported by the cytoskeleton - a network of filamentous proteins distributed throughout the cell. However, the time scales of these processes varies from seconds to minutes and therefore cannot fully account for the instant and continuous membrane remodelling required for many physiological processes.

In this study, the researchers showed that even before the onset of active membrane remodelling, membranes instantly respond to the physical forces that cause cells to change shape, and are remodelled through a passive, mechanical process.

This discovery was made when two types of mechanical stimuli were used to induce cell shape changes. Specifically, the cells were stretched by submitting them to a fixed amount of linear strain, or swollen by being exposed to a hypo-osmotic medium. In both cases, a rapid release of tension, either by stretch release or re-exposure to isotonic medium respectively, resulted in the formation of numerous, small, membrane reservoirs which were quickly resorbed. The nature of membrane reservoirs appeared different depending on the type of mechanical stimuli, for instance, tubular invaginations resulted from stretch release whereas osmotic shock caused larger vacuole-like dilations (VLDs).

The formation of these membrane reservoirs indicated the activation of a rapid membrane remodelling process in response to stress. The authors observed that this is a passive process as it did not require energy or the cytoskeleton, and resembled the response of a synthetic lipid bilayer to similar mechanical stimuli.

This study revealed the existence of a mechanical membrane remodeling process that can instantly respond to cell shape changes. Membrane reservoirs are created in order to prepare the cell membrane for a rapid increase in membrane area. This explains how large membrane requirements for certain cellular processes like cell spreading can be rapidly met. As reservoirs can store and release membrane upon subsequent stretch cycles, they may function to support the constant requirement of membrane remodeling in vital processes where cells undergo continual cycles of stretch and relaxation, such as breathing or as the heart beats.



Membrane reservoirs enable changes in cell size and shape

Illustration showing the formation of membrane reservoirs following tension release in cells subjected to physical stress.

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NILS GAUTHIER

Former Senior Research Fellow at the Mechanobiology Institute until 2015. In January 2016 he joined FIRC Institute of Molecular Oncology in Milan, Italy. There his research group is focused on using interdisciplinary approaches to investigate Mechano-Oncology.

REFERENCE:
Kosmalska AJ et al. Physical principles of membrane remodelling during cell mechanoadaptation. Nature Communications. (2015) 6:7292. doi: 10.1038/ncomms8292.

CELLULAR INSTRUCTIONS FOR TISSUE REPAIR

Written by Sruthi Jagannathan.

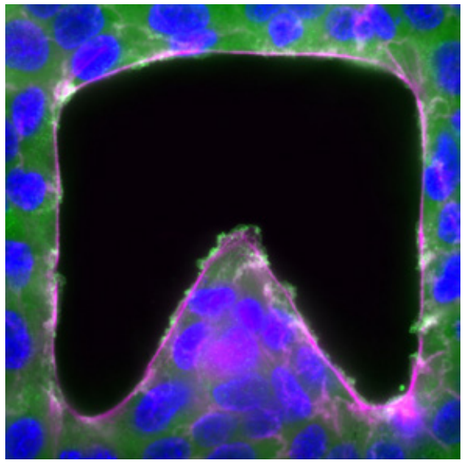
A collaborative study led by Senior Research Fellow Andrea Ravasio and Prof. Benoit Ladoux, described a universal mechanism that regulates forces during epithelial tissue repair. This work was published in Nature Communications.

How tissue shape regulates wound repair mechanisms

The epithelial tissue, or the epithelium, is one of four major types of tissue that lines the surfaces of all organs and hollow spaces in our body. The epithelium protects the organs from damage and maintains the body in a state of balance by allowing a selective in-and-out passage of substances. Proper function of the epithelium requires an intact layer of epithelial cells. During the lifetime of an organism, gaps or holes of different sizes and shapes are introduced into this intact epithelium. They may appear as a consequence of natural biological processes such as embryo development when cells move around and rearrange to establish body patterns, or during cell turnover in adult tissues, when dead cells are cleared away by neighbouring healthy cells. In addition, injury or disease may also lead to wounds or ulcers in the tissue. In either case, any gap in the tissue needs to be sealed so that the normal functioning of the tissue is restored. In the likelihood of an open wound or gap causing complications such as infections, inflammation, or even cancer, our body has developed two major repair mechanisms whereby cells surrounding the gap collectively move in and seal the open spaces completely.

To do so, cells either put forth finger-like protrusions called lamellipodia to crawl along the underlying surface or form an interconnecting belt or cable of actin filaments and myosin proteins. When this cable contracts, it pulls the cells closer in a coordinated fashion, similar to the action of drawing a purse-string. However, the extent to which either mechanism contributes to tissue repair is known to depend on several factors such as the gap geometry, gap size or the presence or absence of an underlying supporting surface.

To determine the impact of tissue geometry on gap closure, a research team led by MBI Senior Research Fellow Dr. Andrea Ravasio and MBI co-Principal Investigator Prof. Benoit Ladoux, in collaboration with scientists from Pierre et Marie Curie University, France, the Institute for Bioengineering of Catalonia, Spain, the Chronic Disease Research Centre, Portugal, the Weizmann Institute, Israel and the University of Cambridge, UK studied the effects of gap shapes on gap closure mechanisms. By using microfabrication techniques to grow epithelial cells around stencils made of an inert polymer, they created gaps of desired shapes within the cell culture.



The boundaries of the gap were either protruding inwards (concave edges) or were extending away (convex edges). Interestingly, the researchers noted that the speed at which cells along the gap edge moved depended on the local curvature. Cells at the convex edge moved in faster than those at the concave edges. Their findings suggested that this could be due to the balance of forces generated by cell movement during the two repair mechanisms. In this case, the crawling movement of cells generate backward forces on the surface that pushes them forward. Alternately, contraction of the actin cable generates forces parallel to the gap edge that push the cells at the concave edges backwards, while the cells at the convex edges are pulled forward. As a result, the two forces act in opposition at concave edges but support each other at the convex edges. This ultimately allows cells at convex edges to move faster. To test the relevance of their findings in a living system, the researchers studied wound repair in flies and found a similar association between wound shape and wound repair.

This study identified a universal mechanism that explains how the geometrical properties of tissue regulate forces and guide cellular movement during physiological processes such as cell turnover, embryo development and wound healing. Cells essentially receive the instructions on how to close a gap by sensing and measuring the shape of the gap itself. Further understanding of how cells do this will help researchers know how to treat chronic medical conditions involving wounds or unsealed gaps as well as designing new substrates to optimize tissue regeneration. As a way of highlighting the significance of this study, one of the images from the publication was chosen as the featured image for the journal issue.

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REFERENCE:
Ravasio A et al., Gap geometry dictates epithelial closure efficiency. Nat Commun. 2015 Jul 9;6:7683. doi: 10.1038/ncomms8683.

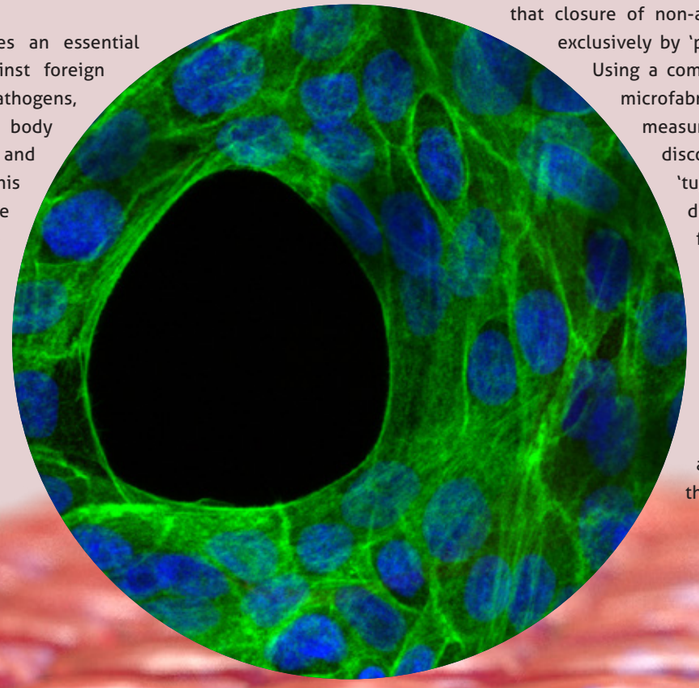
FORCING WOUNDS CLOSED

Written by Andrew Wong. Illustrations by Diego Pitta de Araujo and Cindy Zhang.

A collaborative study led by scientists Prof. Chwee Teck Lim, and Prof. Benoit Ladoux revealed the mechanical forces that drive epithelial wound healing in the absence of cell supporting environment. This research was published in Nature Communications.

A cellular tug-of-war mechanically drives gap closure

Skin not only provides an essential protective barrier against foreign materials and pathogens, but it also helps the body retain various fluids and electrolytes. When this barrier is damaged, the consequences can be devastating. Ulcers, bleeding and bacterial infections may result and the chances of these occurring increases the longer wounds remain open.



Microscopy image courtesy of Vedula Sri Ram Krishna

Fortunately, epithelial cell sheets are self-repairing. The moment the integrity of the barrier is compromised, cellular mechanisms are initiated to close the gap. Cells begin crawling forward, and contractile cables are formed in the cells surrounding the wound to help pull the gap closed. For several years scientists have been learning much about how cells coordinate these processes and repair wounds quickly. In most cases, the healthy skin cells responsible for carrying out wound repair rely on a supporting layer underneath them. This layer comprises sticky proteins, and is known as the extracellular matrix (ECM), which provides support for them to adhere to and crawl over.

However, in cases of chronic or severe wounds, the underlying layers could also be damaged. Surrounding cells could also be unable to replace the ECM proteins. Yet the repair of these gaps, known as non-adherent gaps, does occur, albeit at a slower rate and with an increased likelihood of infection or other complication. So the question remained; how do cells close gaps in protective epithelial barriers where the underlying layers are also damaged or the ECM eroded?

This question was the focus of a study led by MBI Principal Investigator Chwee Teck Lim and Co-Principal Investigator Benoit Ladoux, along with MBI PI Yusuke Toyama. Published in Nature Communications, their findings reveal that closure of non-adherent gaps is driven exclusively by 'purse-string contraction'. Using a combination of cell culture, microfabrication and force measurements, the scientists discovered that a cellular 'tug-of-war' at the gap edge drives the mechanical forces responsible for gap closure.

The cells at the edge of the non-adherent gap are still attached to the ECM. These cells then spread themselves out as far as possible towards the centre of the gap. Measuring the direction of force revealed that these cells are actually pushing away from the gap. While this may sound counter-intuitive, it actually stabilises the cells, in a similar manner to a cantilever bridge, where support at either end anchors the extension of the bridge into space until two sides eventually meet in the middle. Once the cells have spread as far as possible into the gap, the contractile 'purse-string' cable forms across the cells, encircling the gap. The force exerted by these cells is reversed and the cells begin to pull each other towards the centre of the gap, continually speeding up the contraction of the protein cable. As the cells move inwards to close the empty space, more contractile cables can reach out over the gap and connect to the other side. These cables can contract rapidly, leading to the formation of a suspended cell sheet over the gap, and complete closure of the wound.

The 'tug-of-war' mechanism identified in this study provides a vivid demonstration of how cells exert directional forces to enhance biological processes. This new knowledge of the mechanical properties of skin and internal epithelial cells may lead to advances in wound repair, especially in cases where the ECM is compromised. With chronic wounds, sores and ulcers a common complication in several diseases, particularly those associated with aging, it is imperative that researchers better understand the mechanisms at play in their repair. This will undoubtedly lead to improved treatments in wound healing.

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REFERENCE:

Vedula et al., Mechanics of epithelial closure over non-adherent environments, Nature Communications, 6, Article number: 6111, doi: 10.1038/ncomms7111

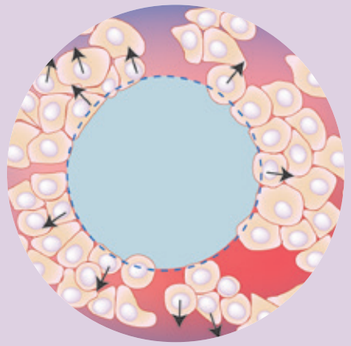


Figure: Forces exerted by the cells surrounding the gap (dotted blue line) extend away at first, then direct in, towards the gap, during contraction of the 'purse-string' cable (red filaments).

FLUID ON SOFT

Written by Andrew Wong.

A collaboration between MBI researchers in Singapore, and researchers from CNRS, Paris Diderot, and Pierre et Marie Curie Universities Paris, in France, revealed how cells can respond to the rigidity of their surrounding environment by adapting their internal mechanical properties. This study reveals that cells adapt their rheology - behaving as a fluid on soft substrates and as an elastic solid on stiff substrates through a large scale remodelling of the cytoskeleton. This study was published in Nature Communications.

External stiffness drives internal organization

During their lifetime, cells encounter environments of various stiffness. These changes affect cell-generated forces and as such, influence their movement, rate of division, and eventual fate. In a classic experiment, stem cells grown on a soft substrate developed into brain cells, yet the same cells became bone cells when grown on a hard substrate. Apoptosis, the process of programmed cell death, is also increased within soft environments. Interestingly, all these important processes depend on the level of cell contractility: cells pull harder on stiffer substrates than on soft ones.

Previous studies have focused on describing how the cell regulates this rigidity-sensing mechanism by local recruitment of specialized protein clusters at adhesion sites. MBI researchers, led by Prof. Benoit Ladoux, together with collaborators from CNRS, Paris Diderot, and Pierre et Marie Curie Universities (Paris, France) revealed that another underestimated mechanism, a large-scale remodelling of the internal cytoskeleton network, also plays an important role. To do so, they used an imaginative inverted experimental setup to observe cytoskeleton reorganization in live cells on substrates of varying stiffness. Cells were grown on a surface of flexible micro-needles whose stiffness can be tuned by changing the height of the needles.

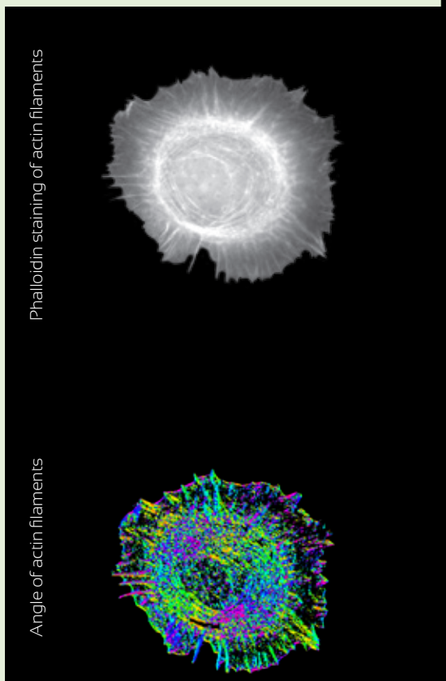
Based on experiments and theoretical modelling, they discovered that the cytoskeleton adapts its rheology, from fluid–like on soft substrates to solid–like on stiff substrates, as well as its internal organization, from disorganized to organized, to dictate the response of the cell to substrate rigidity. On a soft substrate, cells experience low surface friction, which leads to increased cytoskeleton flow towards the cell centre, and thus to a fluid-like state. These circular, fluid-like cells do not polarize.

As the substrate stiffness increases, the cell substrate friction also increases. This leads to the assembly of large and stable contractile bundles of cytoskeletal filaments, which organize into locally aligned clusters, while remaining disorganized at the whole cell level. These contractile bundles, which are rigid, lead to a solid-like state of the cell.

When the substrate stiffness is further increased, these contractile bundles remodel themselves and align in a single preferential direction, becoming organized at the whole cell level, and this leads to establishment of cell polarity. This evolution from a disorganized to organized state in a biological system is similar to that observed in well-known physical systems such as liquid crystals.

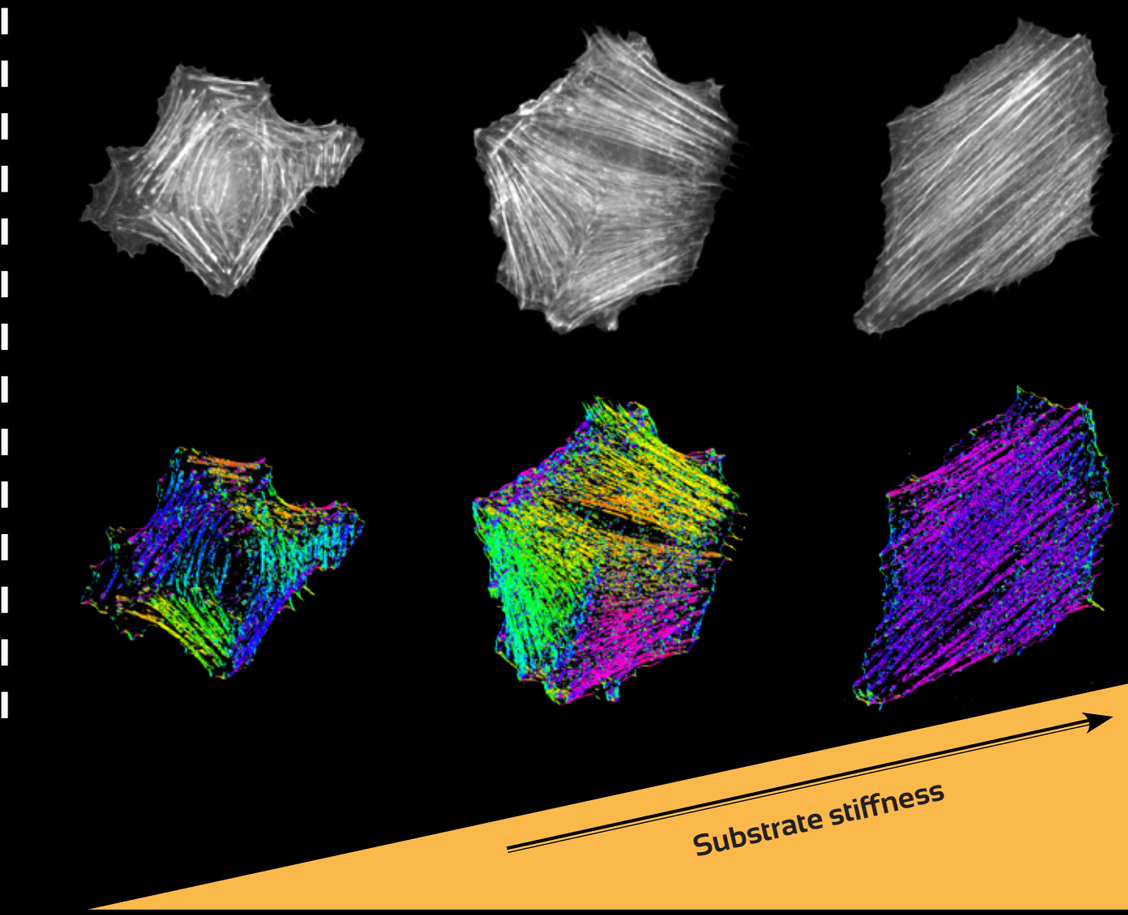
Whole cell mechanosensing

This global sensing mechanism may explain various cellular responses to rigidity, such the preferential migration of tissue cells towards stiffer regions or even the increase of the apoptotic rate on softer substrates. Consequently, these findings could have important implications in pathological situations such as cancer where tumours appear as a rigid mass compared to healthy tissues, but also in physiological processes such as cell differentiation where cytoskeleton organization and forces may favour different outcomes.



Microscopy images courtesy of Mukund Gupta

SOLID ON STIFF



Large-scale cytoskeletal remodelling in response to environmental stiffness

On a soft surface, the actin cytoskeleton is fluid and disorganized, with filaments pointing in all directions. As substrate rigidity increases, the filaments assemble into locally organized clusters which impart a solid-like rheology to the cell. Further increase in substrate stiffness leads the actin filaments to organize in one direction across the whole cell.

REFERENCE:
Gupta et al., Adaptive Rheology and ordering of cell cytoskeleton governs matrix rigidity sensing, Nature Communications, 2015 doi: 10.1038/ncomms8525

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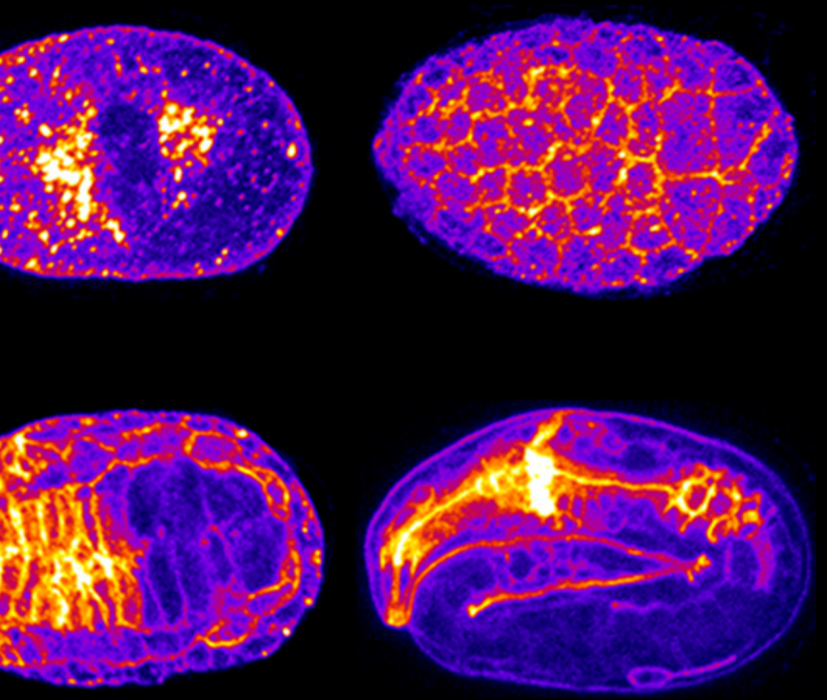
LIPID ANCHORS SUSTAIN LIFE

Written by Lakshmi Ramachandran and Andrew Wong.

A research project led by Asst. Prof. Ronen Zaidel-Bar, and published in March 2015, revealed that glycolipids which anchor certain proteins to the cell membrane also play a vital role in stabilizing the membrane, especially against mechanical stress that arises during embryonic growth and development. This study was published in PLoS Genetics.

All living cells have a membrane around them, forming a protective barrier that helps contain the various cellular components and prevent entry of foreign material into the cell. The cell membrane is made of lipids and proteins, contributing to membrane structure and function. Some membrane proteins integrate within or span the lipid bilayer structure of the membrane, while some associate with the membrane exterior either directly or through addition of a Glycosylphosphatidylinositol (GPI) lipid anchor. It is well known that GPI anchors are critical for the normal growth and development of an embryo. However, the question of how they play a role in embryonic development remains unanswered, as mutants of GPI anchor biosynthesis required to investigate the role of GPI anchors in living organisms typically die at a very early embryonic stage, preventing further studies.



However, Dr. Yemima Budirahardja and Thang Doan, from the lab of Asst. Prof. Ronen Zaidel-Bar, MBI, overcame this challenge by using a GPI anchor biosynthesis mutant of the worm *Caenorhabditis elegans* with a partial loss of function. They found that mutations which reduce the synthesis of GPI anchors weaken and disrupt the epithelium, a major tissue in our body that makes up the skin, as well as the respiratory, digestive and reproductive systems. In the mutant embryos, breaches in the epithelium lead to cyst formation in the intestine and spillage of cells out of the skin. This means that GPI anchors are necessary to maintain the integrity of the epithelium and prevent embryonic lethality.

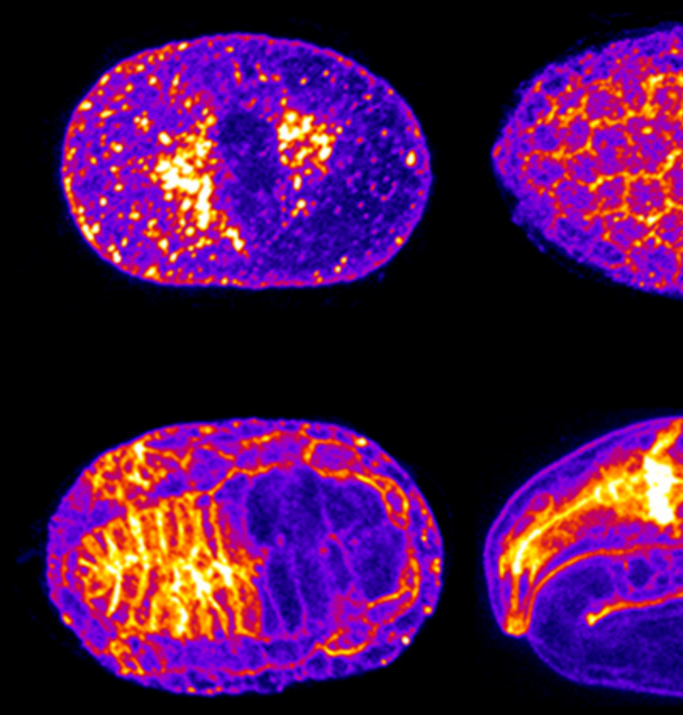
The mechanism by which the GPI anchors exert a stabilizing force to the apical cell membrane is not yet understood, but one intriguing possibility is that they do it through an indirect connection to the cytoskeleton that lies beneath the membrane. When this connection is disrupted, the cell becomes more susceptible to external forces and mechanical stress. These stresses occur regularly during development, as the embryo divides and reorganizes, or may arise internally, such as intestinal expansion and contraction during digestion.

Intriguingly, Budirahardja and colleagues discovered that reinforcing the links between the cytoskeleton and the membrane from within the cell can protect against these mechanical stresses, even in the absence of GPI anchors.

GPI-anchors protect embryos from mechanical stresses by strengthening epithelial integrity

This study suggested a basic underlying mechanism for GPI anchor function in maintaining epithelial cell integrity. Minor mutations in GPI anchor biosynthesis have severe consequences in humans, resulting in diseases such as paroxysmal nocturnal hemoglobinuria, where 'leaky' red blood cells are attacked by the body's own immune system, and the congenital disorder Mabry syndrome, which is characterized by intellectual disabilities and abnormalities in the renal and digestive system. Understanding the role of epithelial integrity in these GPI anchor diseases could provide new insight into how these diseases manifest and progress, as well as inspire potential therapies based around strengthening the link between the cytoskeleton and the apical membrane.

Tissue Mechanics



ABOUT THE RESEARCHER: RONEN ZAIDEL-BAR

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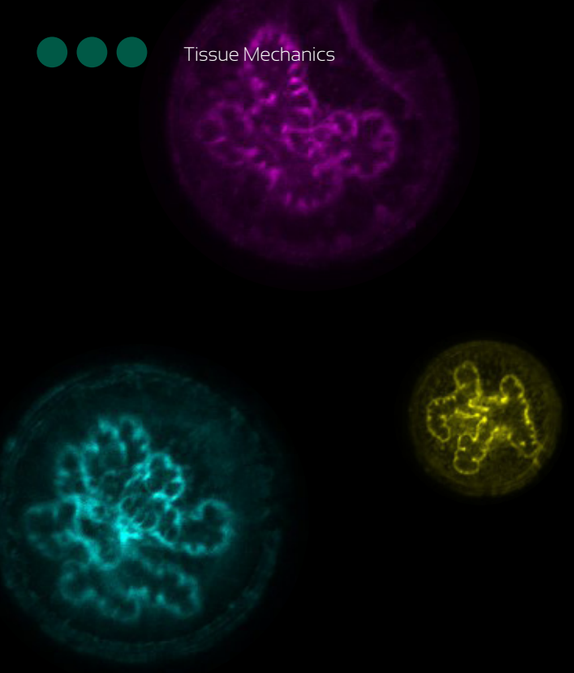
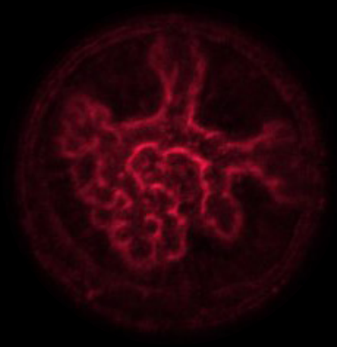
REFERENCE
Budirahardja et al., Glycosyl Phosphatidylinositol Anchor Biosynthesis Is Essential for Maintaining Epithelial Integrity during Caenorhabditis elegans Embryogenesis, PLoS Genetics, 25 March 2015, doi: 10.1371/journal.pgen.1005082



SHAPING LUMENS BY FORCE

How extracellular matrix scaffolding guides lumen elongation

Written by Sruthi Jagannathan.



A team of scientists from Singapore and France revealed the underlying mechanism for the formation and growth of a fundamental type of tissue-epithelial tubes. Their study suggested that the shape and size of the epithelial tubes are governed by the mechanical forces that arises from the interaction of cells with the supportive extracellular matrix (ECM) that surrounds them. This work was published in Nature Cell Biology.

Extracellular matrix organization guides lumen morphology

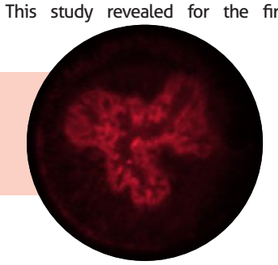
All the major organs in our body, such as the blood vessels, lungs, kidneys, liver, pancreas and the intestine, are formed of an extensive network of tubes. They function as biological pipelines for the transport of life-sustaining liquids, gases or macromolecules from one site in the body to another. Depending on the organ in which they are formed or the specific function that they perform, tubes vary greatly in size and shape. Defects in tubular architecture have been linked to a number of diseases such as atherosclerosis and polycystic kidney disease.

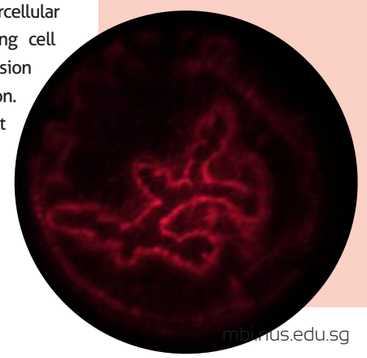
The tubes enclose hollow spaces called lumens and are primarily composed of a single layer or multiple layers, of epithelial cells. As an important prerequisite for tube formation, epithelial cells become asymmetric or 'polar', acquiring structurally and functionally distinct ends or surfaces. Following this, cells undergo shape changes and organize around a central lumen, with their apical (top) surfaces facing the lumen, the basal (bottom) surfaces interacting with the underlying tissue and the lateral (side) surfaces in close contact with the neighbouring cells. The vast majority of studies on tube formation have focused on understanding the molecular mechanisms leading to cell polarization and subsequent cellular mechanisms that drive the formation of lumens. However, factors that regulate the shape, size and the directional elongation of lumens into tubes, remain unclear.

A recent collaborative study led by Assoc. Prof. Virgile Viasnoff, Principal Investigator at the Mechanobiology Institute, NUS and CNRS (France) and Prof. Hanry Yu, Principal Investigator at MBI and the Institute of Bioengineering and Nanotechnology, A*STAR, Singapore, aimed to address these key questions. Studying the formation of 'bile canaliculi', which are lumens formed between the contacting lateral surfaces of two liver cells, the scientists had adopted a 'minimal organ approach'. This involved culturing two liver cells (hepatocytes) that can act as a functional organ unit on artificial membranes fabricated with microwell patterns. The microwells are coated with an ECM protein called fibronectin that promotes cell binding and creates growth conditions identical to the microenvironment found inside cells. By coating the microwells in different patterns, the scientists altered the organization of ECM around cells and compared the morphologies of the bile canaliculi and the direction of their growth. Surprisingly, they observed that lumen shape was controlled by the three dimensional organization of ECM around cells. Furthermore, lumens showed a preference to elongate towards the free surface of the cell, away from the ECM.

Following up with a series of experiments to understand the role of ECM in determining lumen shape and elongation, the researchers proposed a mechanical basis for the regulation of lumen morphology. According to their model, forces arising from the adhesion of cells to the ECM influences the force balance inside cells and creates an intercellular force (force between two contacting cells) gradient. The lumen elongates along the direction of minimal force, as higher intercellular force would squeeze the contacting cell surfaces together and prevent extension of the lumens in that direction. This study revealed for the first time, that the interaction between cells and the ECM can control and direct the mechanical tension between cells. This mechanical tension directly influences the elongation direction of the intercellular lumen. This mechanical guidance of lumen morphology is responsible for varied lumen shapes and sizes, formed under different microenvironmental conditions. A deeper understanding of the mechanical principles, in addition to molecular and cellular mechanisms that underline epithelial tube formation is essential for developing improved therapies for diseases such as cholestasis, atherosclerosis, and polycystic kidney disease that arise from defects in tube architecture.

REFERENCE:
Li et al., Extracellular matrix scaffolding guides lumen elongation by inducing anisotropic intercellular mechanical tension. Nat Cell Biol. 2016 Mar;18(3):311-8. doi: 10.1038/ncb3310





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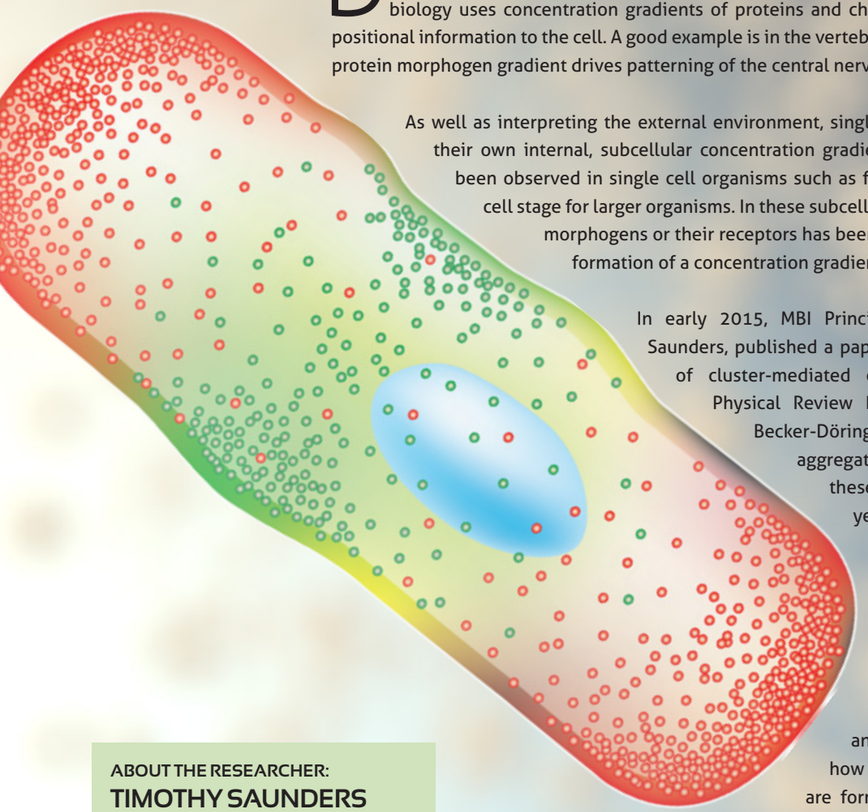
CLUSTERING DRIVES FORMATION OF SUBCELLULAR MORPHOGEN GRADIENTS

Written by Andrew Wong. Illustrations by Cindy Zhang and Diego Pitta de Araujo.

During development, stem cells determine their fate based on their location and surroundings. In the same way as the increasing depth of a swimming pool informs whether you stand or float, biology uses concentration gradients of proteins and chemicals called morphogens to provide positional information to the cell. A good example is in the vertebrate embryo, where the sonic hedgehog protein morphogen gradient drives patterning of the central nervous system.



As well as interpreting the external environment, single cells can also create and respond to their own internal, subcellular concentration gradients. These subcellular gradients have been observed in single cell organisms such as fission yeast, bacteria and at the single cell stage for larger organisms. In these subcellular systems, clustering of the signaling morphogens or their receptors has been observed, but the role of clustering in formation of a concentration gradient is still unclear.

In early 2015, MBI Principal Investigator, Asst. Prof. Timothy Saunders, published a paper describing a new theoretical model of cluster-mediated concentration gradient formation in Physical Review E. A/Prof Saunders started with the Becker-Döring equations describing the processes of aggregation and fragmentation. By combining these equations with experimental data of yeast morphogen gradient formation and incorporating biophysical constraints, he was able to refine and validate a mathematical model that accurately produced robust concentration gradients.

This new mechanistic model provides an updated framework for understanding how subcellular morphogen gradients are formed. Comparing this with our current knowledge on the formation of embryonic morphogen gradients could reveal new insights into development.

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REFERENCE:
Saunders TE. Aggregation-fragmentation model of robust concentration gradient formation, Phys. Rev. E, 91, 5 February 2015, http://dx.doi.org/10.1103/PhysRevE.91.022704,).

MICROFLUIDICS FOR LIQUID BIOPSIES

Written by Sruthi Jagannathan. Illustration by Chun Xi Wong.

A team of scientists led by Prof. Chwee Teck Lim developed a miniaturized device that can rapidly and efficiently detect and isolate cancer cells from whole blood. Based on an emerging technology called microfluidics, the device adopts a simple, highly non-invasive methodology while remaining low-cost, and provides an ideal platform for point-of-care cancer diagnostics and treatment.

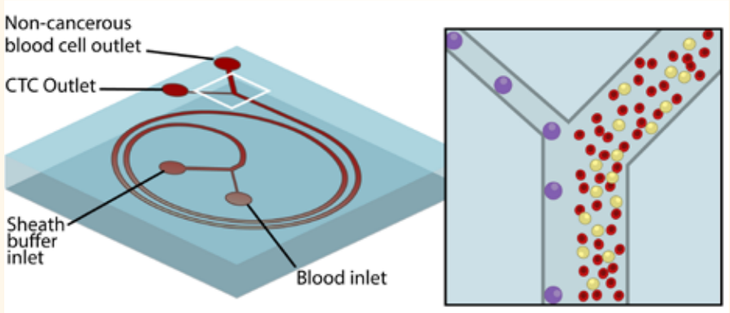
A tool for point of care cancer diagnostics and treatment

Microfluidics is characterized by the precise manipulation of fluid within sub-millimetre channels, at which scale the effect of physical properties, such as capillary force and surface tension, on fluid behaviour is considerably greater than at the macro-scale. Rapid advancements in the technology has expanded biomedical research through the development of lab-on-a-chip devices for a number of biochemical analyses such as cell separation, DNA and protein analyses, and PCR amplification. By applying microfluidics to the detection and isolation of cancer cells, the research team developed a spiral microfluidic chip that enables an ultrafast and efficient, size-based separation and isolation of circulating tumour cells (CTCs) from whole blood. As the name implies, circulating tumour cells are cancer cells that get disseminated in the peripheral bloodstream, and over the course of the disease, invade other body parts, where they proliferate to form secondary tumours. The ability of cancer cells to spread aggressively is known as metastasis and is responsible for the majority of cancer related deaths.

CTCs are extremely rare cells that start occurring in the early stages of cancer, and their levels rise steadily as the disease progresses. Therefore, unlike tissue biopsies that can be used only as a diagnostic tool and can only be performed once before and after cancer treatment, liquid biopsies involving the isolation and enumeration of CTCs can be used for real-time diagnosis as well as for assessing metastatic risk, prognosis and treatment efficacy in patients during therapy. Conventional cell separation methods developed so far for the isolation of CTCs have been limited by disadvantages such as poor CTC detection sensitivity, high degree of damage to isolated CTCs or contamination with other blood cells, and their inability to process large volumes of blood.

The spiral microfluidic chip described in this study successfully overcomes the challenges involved in the isolation and characterization of such rare blood cells. The chip is made of a silicon-based organic polymer and consists of two loops of microchannels that are etched using a patterning technique called soft lithography. Once the blood is pumped through the inlet at an optimized flow rate, the spiral design and the dimensions of the channels create hydrodynamic forces that cause the larger CTCs to flow along the inner wall of the channel, whereas the smaller, non-cancerous blood cells flow along the outer wall. By collecting the separated blood cell fractions via different outlets, the technique ensures negligible contamination of CTCs with other non-cancerous blood cells, and an almost 100% CTC detection sensitivity. The device also has the ability to continuously process blood samples, leading to a high recovery of CTCs. The small size of the microfluidic device allows multiple units to be stacked together that can further increase the diagnostic output.

Beside their economic feasibility and enhanced performance, the spiral microfluidic chip offers additional advantages over other cell separation systems, such as portability, the need for low volumes of sample and ultra-fast processing time. The methodology thus has great potential to be translated into a highly reliable and patient friendly clinical test for the diagnosis, prognosis, management and treatment of cancers. The recovery of highly pure fractions of CTCs also enables its use for downstream DNA and protein analyses, which can reveal vital information on cancer biology and serve as research platforms for the discovery of new cancer drugs and their pre-clinical testing. A detailed protocol describing the principle of operation, design, fabrication and applications of the spiral microfluidic chip has was published in Nature Protocols.



REFERENCE:
Warkiani ME et al., Ultra-fast, label-free isolation of circulating tumor cells from blood using spiral microfluidics. Nat Protoc. 2016 Jan;11(1):134-48. doi: 10.1038/nprot.2016.003

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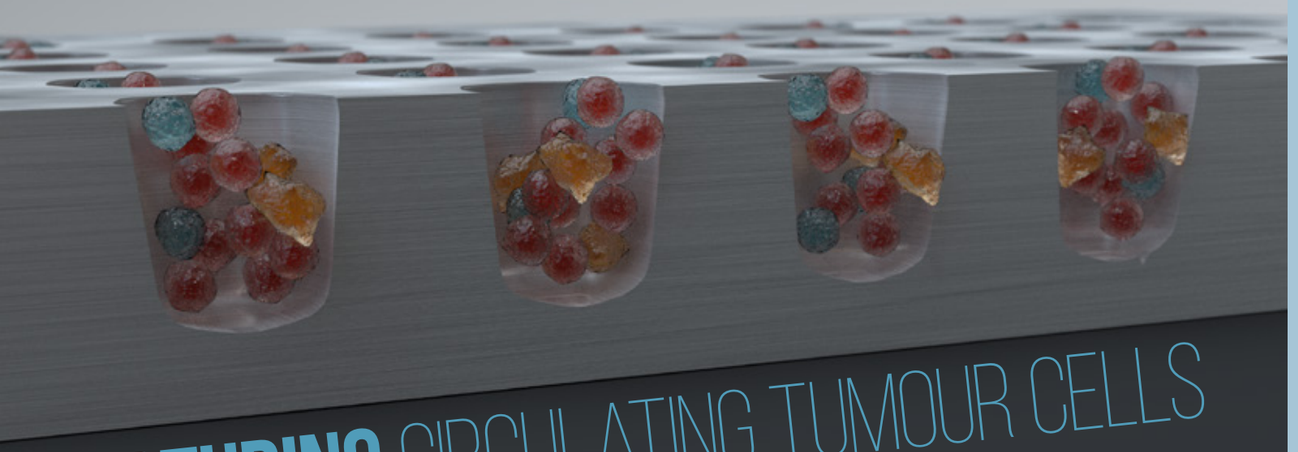


CULTURING CIRCULATING TUMOUR CELLS

Written by Sruthi Jagannathan. Illustration by Diego Pitta de Araujo and Cindy Zhang.

Collaboration between scientists, clinicians and engineers from the Mechanobiology Institute, the Cancer Science Institute of Singapore, and the National University Hospital at the National University of Singapore, along with researchers from the Singapore-MIT Alliance for Research and Technology (SMART) centre has led to the development of a novel technique for culturing circulating tumour cells (CTCs) that could be used to predict cancer treatment outcome. This study was published in Oncotarget.

Cancer is among the leading causes of death in Singapore today. More than a hundred types of cancers have been identified, each with distinct characteristics and treatment challenges. Cancer results when genetic anomalies cause a few healthy cells to divide uncontrollably into a mass of abnormal cells called a tumour. As the cancer develops, some cells gain the ability to escape from the tumour, invade the bloodstream and subsequently squeeze out from the blood vessels to attach themselves to other parts of the body. This process, known as 'metastasis', is associated with poor prognosis and high mortality rates.

The cells that manage to slip away from the tumour and enter the bloodstream are termed circulating tumour cells (CTCs). CTCs can be found even in the early stages of cancer and techniques that detect CTCs from patient blood can help in understanding cancer progression as well as predict patient response to cancer treatment. However, most conventional methods used to isolate CTCs from whole blood have lacked efficiency, as CTCs comprise of many sub-populations and occur at low frequencies in blood. Furthermore, the procedures used to exclusively identify certain sub-populations of CTCs from whole blood, are often unable to isolate all of the CTCs.

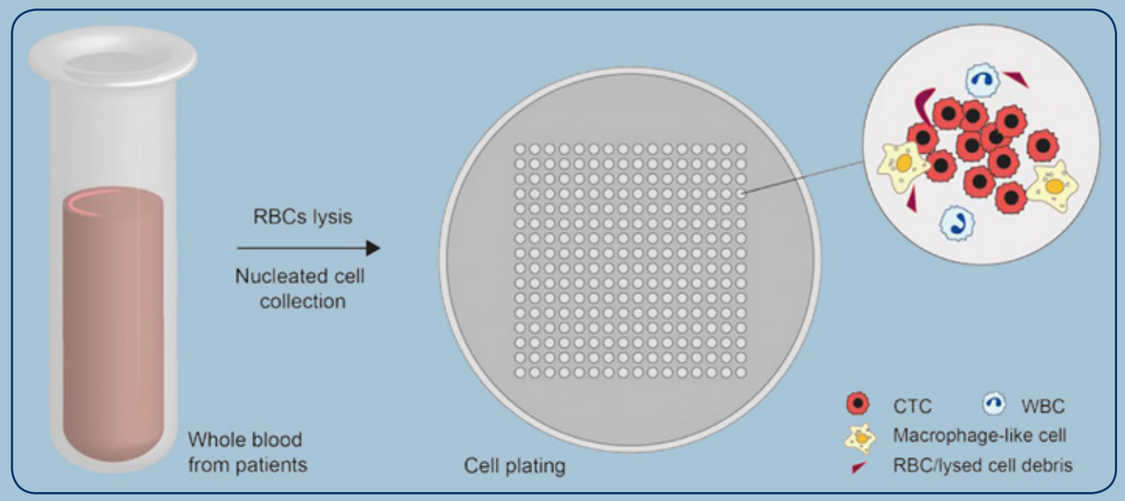
In an attempt to overcome these setbacks, MBI researchers developed a novel methodology for efficiently culturing CTCs from whole blood. In this method, CO_2 lasers were used to engrave microwell patterns on a petri dish and whole blood samples from patients with early stage or metastatic breast cancer, which were treated beforehand to exclude the red blood cells, were deposited into the microwells.

Most of the CTCs were found to be intact following this pre-treatment. The geometry of the microwells, the non-sticky nature of their surfaces, and the oxygen-deficient growth conditions provided an ideal environment for tumour cell growth. Other non-cancerous cells, such as white blood cells, gradually underwent cell death and were removed from the culture over time. Remarkably, the authors discovered the formation of cell clusters after two weeks of culture and the clusters primarily consisted of cells with characteristics of CTCs. Mechanistically, the cells increasingly resembled cancer cells with higher invasive characteristics, while their genetic profiles revealed cancer-causing mutations.

Tests were conducted on over 220 clinical samples from patients with both localized and metastatic breast cancers, and a success rate of over 60% in culturing CTCs was achieved. In patients undergoing cancer treatment, the authors observed that the formation of CTC clusters progressively declined with increase in treatment duration. This could be due to the clearing away of cancer cells from patient's body, in response to treatment. In line with this, persistent cluster formation post-treatment suggests a poor response and poorer survival time.

The chance of getting CTCs in a blood sample is akin to trying to find a hundred people in a world of seven billion

The ability to capture and efficiently grow CTCs from a blood sample will provide researchers and clinicians valuable tools to investigate the best therapy options for the patient. This study described a novel methodology to efficiently culture CTCs from patients with early stage or metastatic breast cancer. The combination of specially designed microwells and biologically suitable conditions provide an ideal microenvironment for CTC growth with many advantages over current methods. Along with other benefits such as shorter culture durations, low quantities of sample needed and a minimally invasive procedure, this technique is well-poised to be an ideal tool for assessing the status of cancer as well as for predicting the efficacy of specific treatment regimes.



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REFERENCE:
Khoo BL. et al, Short-term expansion of breast circulating cancer cells predicts response to anti-cancer therapy. Oncotarget. doi: 10.18632/oncotarget.3903

SINGLE OBJECTIVE LIGHTSHEET MICROSCOPY FOR 3D SUPER-RESOLUTION IMAGING

Written by Pui Yee Loh. Illustration by Chun Xi Wong.

Recent advances in microscopy have allowed scientists to image biological samples in 3D for extended periods of time, without causing damage to the sample. Combined with super-resolution imaging, this means that the activity of single proteins can be followed within individual cells or tissues, providing new insight into protein function, and importantly, how protein dysregulation can lead to disease. Unfortunately, these imaging techniques are still prohibitively complicated and expensive for most labs.

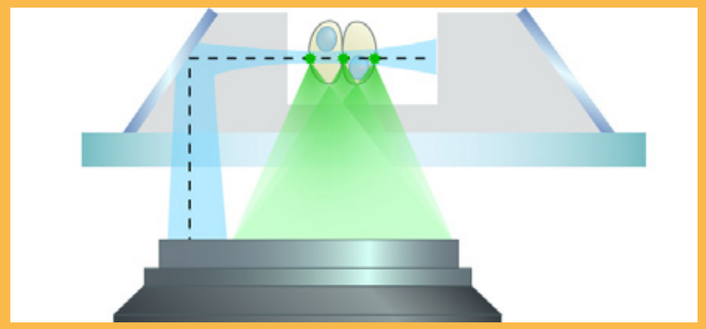
Despite recent progress in the development of super-resolution microscopy only a few techniques, such as total internal reflection fluorescence (TIRF) illumination and interferometric photoactivated localization microscopy (iPALM), enable single-molecule imaging. These techniques are limited to capturing 2D images near the surface of the glass coverslip, or 3D images within the first micrometre (μm) of the coverslip. For reference, a typical human skin cell is 30 μm in height. Selective Plane Illumination Microscopy (SPIM) is a technique that enables 3D super-resolution imaging of thicker samples at a single-cell level. This technique selectively illuminates a single plane of the specimen by directing a focused light sheet from one side while capturing the fluorescence signal through a second objective positioned perpendicularly to the light sheet. A 3D image is reconstructed from collected images of individual cell sections. However, this approach requires a complicated 2-objective system and special sample holder which makes it incompatible with standard microscope systems.

Innovative microfabrication leads to a new microscopy method

With the aim of providing a simple, yet versatile microscopy technique that can identify single proteins anywhere within a cell, and allow cellular organization to be assessed in 3D, researchers at the Mechanobiology Institute, and a CNRS team in Bordeaux, France developed an improved SPIM technique that requires only a single objective, called soSPIM. This new technique was described in Nature Methods.

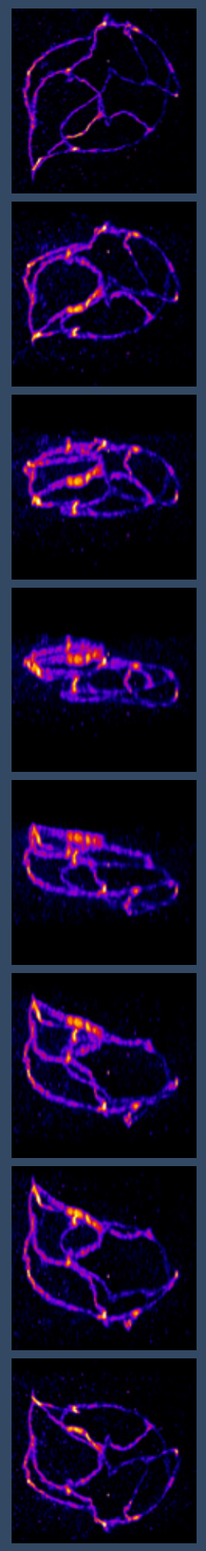
This technique, developed by MBI Principal Investigator, Assoc. Prof. Virgile Viasnoff, utilizes an array of micromirrored wells that are produced via microfabrication. Each mirror, which is inclined at precisely 45°, serves as both a means to direct the excitation beam, and also to hold the sample. Together with a beam steering add-on unit, these micromirrors allow for both the excitation beam, and fluorescence signal, that is viewed through the microscope, to pass through a single standard, objective lens. The soSPIM technique exhibited fast response and good sectioning capability for 3D imaging of a whole cell up to 30 μm above the coverslip. It was also able to identify single proteins, deep within the cell.

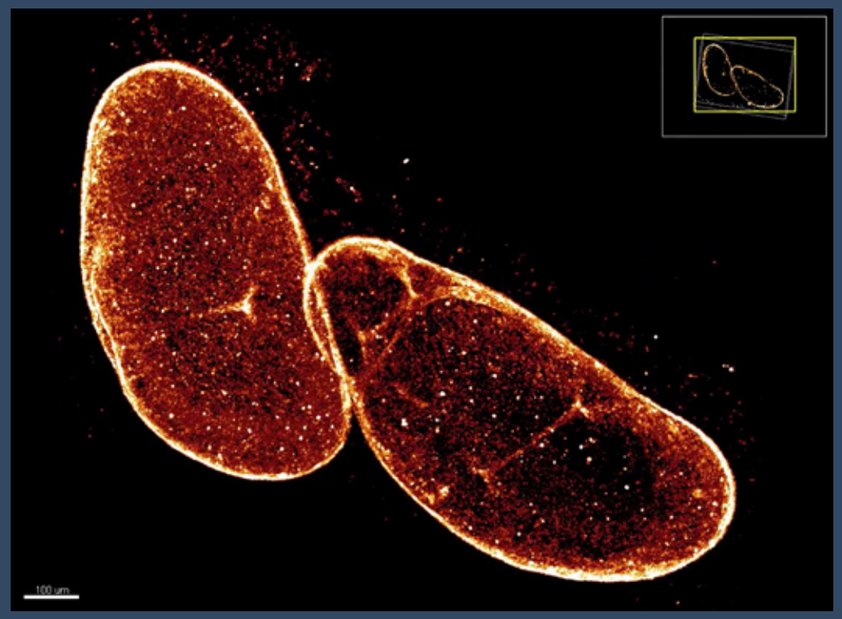
With the microfabricated mirror and sample holder being produced independently of the microscope system, this technique is compatible with standard inverted microscopes and high numerical aperture immersion objective lenses. This will provide more researchers the ability to monitor the activity of single proteins on their existing microscope systems. Protein dysregulation may result in changes to any part or process within the cell. Being able to see these changes is crucial for researchers to fully understand why certain disease states arise. This means being able to visually observe the whole cell in all three dimensions as well as at the single-molecule level. The development of the soSPIM technique not only makes these needs attainable, but does so using conventional inverted microscope technology.



soSPIM schematic

1. Specially designed microfabricated wells contain a chamber for the sample, and are flanked by mirrors inclined at precisely 45°.
2. The excitation beam from the objective (blue) is reflected off the mirror to create a light sheet across the sample. Steering the beam along the mirror enables sectioning of the sample in three dimensions.
3. The emitted fluorescence (green) is collected by the same objective.

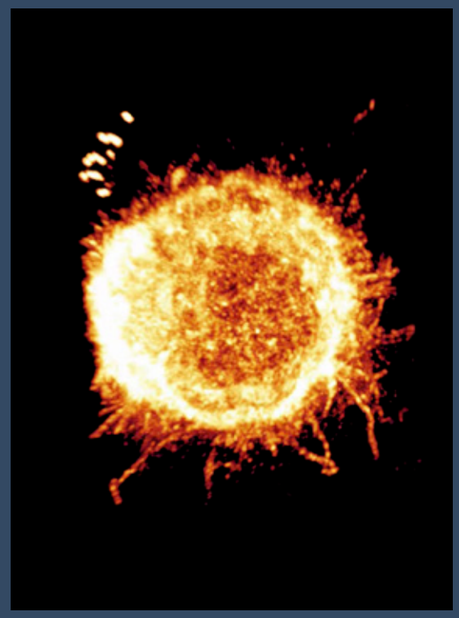




soSPIM imaging

Representative images captured using soSPIM technology demonstrating a wide range of capabilities including super-resolution single molecule detection, live imaging of whole-embryos, and 3D optical sectioning.

Images provided by Dr. Rémi Galland, Interdisciplinary Institute for Neuroscience, Centre National de la Recherche Scientifique (CNRS) and the University of Bordeaux.



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REFERENCE:
Galland et al., 3D high- and super-resolution imaging using single-objective SPIM. Nature Methods, 11 May 2015, doi:10.1038/nmeth.3402

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