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Advances in Science

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FEATURES

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RESEARCH FEATURES

Advances in Science

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On the cover: The mother puts in instructions onto the baby's DNA for turning on specific genes at a specific time. This ensures the proper development of the baby in the womb. [Image generated using an artificial intelligence tool]

Hidden hunger: Exploring the role of fermentation

Fermentation has the potential to enhance the nutritional value of food

Introduction

As agri-food systems become increasingly fragile due to crises such as COVID-19, climate change, African swine flu, forest fires and droughts, there is mounting pressure to establish a global food system that is resilient to future catastrophes. Internationally, there is a growing focus on food security to ensure food availability, accessibility and affordability to the global population. Carbohydrates, fats and proteins (macronutrients) are the primary components of our diet, and their adequate consumption is essential to prevent the population from experiencing "total hunger", where individuals experience a complete lack of food and are unable to meet their basic nutritional needs. To improve the productivity of the agri-food system, food manufacturing side-stream valourisation is increasingly adopted to improve food production and contribute towards a circular food supply chain. However, within the concept of food security, there is a less discussed issue known as "partial or hidden hunger", characterised by micronutrient deficiencies that pose significant global health challenges.

Micronutrients encompass vitamins and minerals that are required in trace amounts for normal body functions. Vitamins A, B_9, B_{12} and D are commonly deficient in the population, while iron, magnesium, iodine and selenium are minerals commonly deficient in populations with dietary restrictions. Vitamin and mineral deficiencies have been associated with various health issues such as ulcers, blurred vision, hair loss and bleeding gums. For example, iodine deficiency was a global concern, resulting in intellectual and development disabilities across several generations. Recognising this, the World Summit for Children aimed to eradicate iodine deficiency by the year 2000, leading to interventions such as the production of iodized salts. By 2013, the USA found that the introduction of iodized salts resulted in an increase of 3.5 points in average national intelligence. While iodine pills exist as supplements, their effectiveness pales in comparison to consumption of dietary iodine. With the rise of sustainable dietary practices such as veganism and vegetarianism, there is a potential risk of "hidden hunger" due to micronutrient deficiencies.

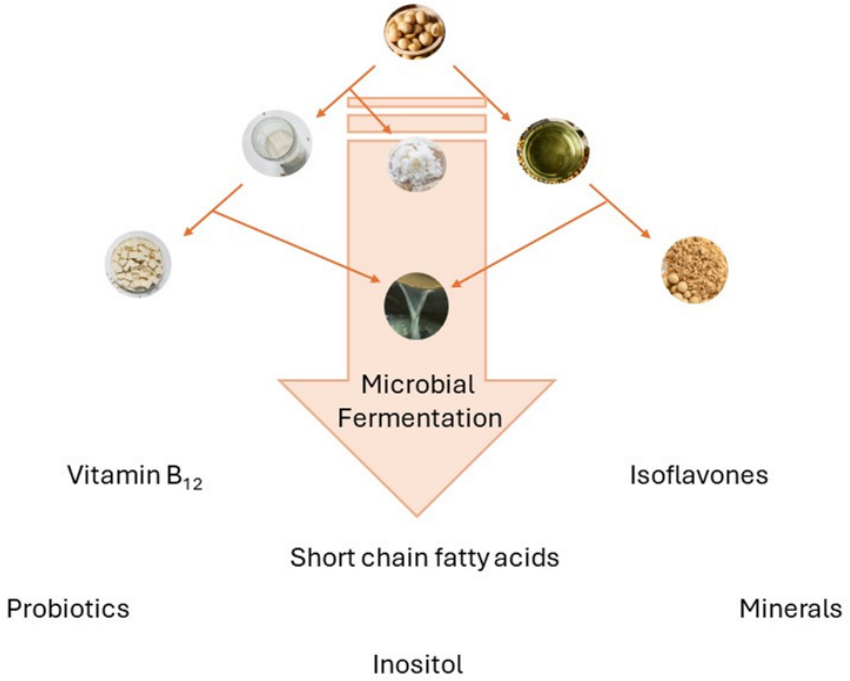

Figure 1: Bioconversion of soybean processing by-products to human nutrient promoting compounds through the process of microbial fermentation.

Vitamin B_{12} biofortification of plant-based foods

A primary concern associated with predominantly plant-based diets is the deficiency of vitamin B_{12}. This vitamin is crucial in the production of DNA and red blood cells. Its absence can lead to symptoms which include nerve problems and vision loss. The daily recommended intake of vitamin B12 is 2.4 μg for adults. This can be easily achieved when consuming animal-derived foods such as dairy, meat and eggs. However, plant-based diets lack this natural source of vitamin B_{12}.

To address the B_{12} deficiency in plant-derived foods, companies have been fortifying synthetic vitamin B_{12} into various plant-based products. However, the synthetic form of Vitamin B_{12}, typically cyanocobalamin, is not as readily absorbed when compared to the natural forms such as methylcobalamin and adenosylcobalamin. To circumvent this issue, we have developed a zero-waste approach, incorporating natural vitamin B_{12} into plant-based foods. Our solution involves probiotic fermentation of manufacturing side-streams to achieve a circular and nutritious food system (see Figure 1).

Soybean is one of the world's leading cultivated pulse crops, known for its high protein content, and soy-based protein is at the helm of plant-based

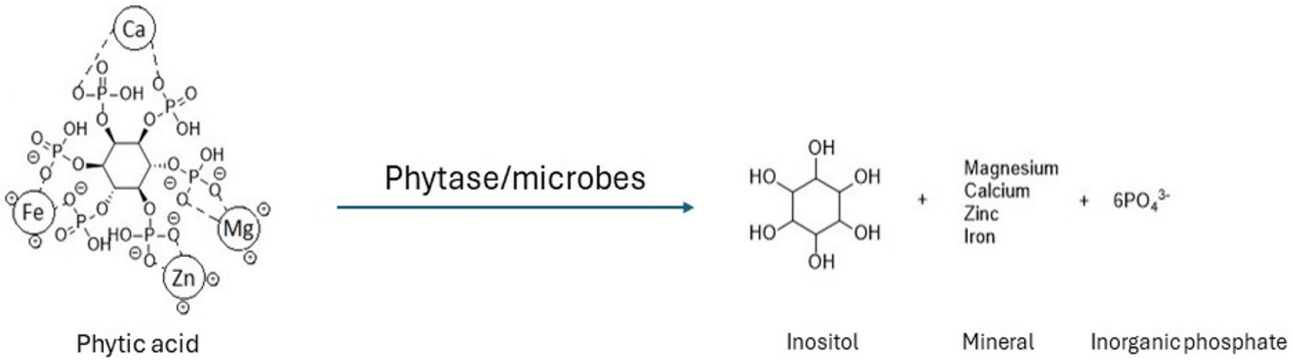

Figure 2: Liberation of minerals from phytic acid to enhance the bioavailability of essential nutrients in food.

diets. It generates significant by-products such as soy pulp and soy whey from the production of soy protein isolate, soymilk and tofu. With Asia dominating soymilk consumption and Singapore producing about 10,000 tons of soy waste yearly, there is a significant opportunity for innovation.

We have recently developed a method to biofortify soy whey with vitamin B_{12} (see Figure 1) [1], addressing a key nutrient gap in plant-based products. Our probiotic-fermented soy whey was able to contain more than 2.4 μg of vitamin B_{12} per 300 mL serving. In addition to the biofortification of B_{12}, our innovation incorporates soy whey with probiotics, short-chain fatty acids, and improves free iron and isoflavones bioavailability.

Anti-nutrients – a complication in plant-based foods?

With growing interest in health-promoting foods, plant-derived food consumption will inevitably rise. Broccoli, kale, soybeans, nuts, whole grains, tea, etc. are all considered very nutritious and healthy foods. Unfortunately, alongside their health benefits, these plant-based foods also contain anti-nutrients. Anti-nutrients, as its name suggests, is a class of compounds that can hinder the absorption of nutrients when consumed. Plants produce anti-nutrients as a self-defense mechanism to deter predators and pests. In broccoli and kale, glucosinolates are present, which can inhibit iodine absorption. Soybeans, nuts and whole grains, contain a combination of lectins, oxalate, phytic acid and saponins which have been linked to reduced calcium, iron and zinc absorption. Tannins in tea have also been shown to interfere with iron absorption, with research showing that these anti-nutritional factors can negatively affect iron absorption by up to 23%.

Efforts to reduce anti-nutrients are well-established, with researchers employing techniques such as cooking, germination, milling and fermentation to improve nutrient bioavailability. Our work on B_{12}-fortified soy whey has enabled us to decrease phytic acid content in both soy pulp and soy whey. Phytic acid, also known as myo-inositol hexakisphosphate, is broken down by phytases into phosphate groups to produce inositol (see Figure 2). This dephosphorylation process not only releases inorganic phosphate, but also other minerals such as iron and magnesium that are bound to the phosphate group, improving mineral bioavailability. Furthermore, inositol, previously known as vitamin B_8, is linked to hormone, neurotransmitters and growth factor regulation.

Next steps

While nutrition is a key parameter in foods, consumers are primarily influenced by their senses of taste and aroma. These sensory aspects are very important factors in consumer purchase decisions. For the circular food system to be a viable reality, foods not only need to be nutritious and healthy, but also tasty and appealing. We will further improve the taste and aroma of our nutritionally boosted ingredients to expand their appeal. By utilising a myriad of food side-streams, or the supplementation of food-grade flavour precursors, we aim to tailor the flavour of the food materials to possibly mimic specific foods such as chocolate or meat sauces.

For more details, please visit: https://www.fst.nus.edu.sg/our_people/faculty-members/liu-shao-quan/

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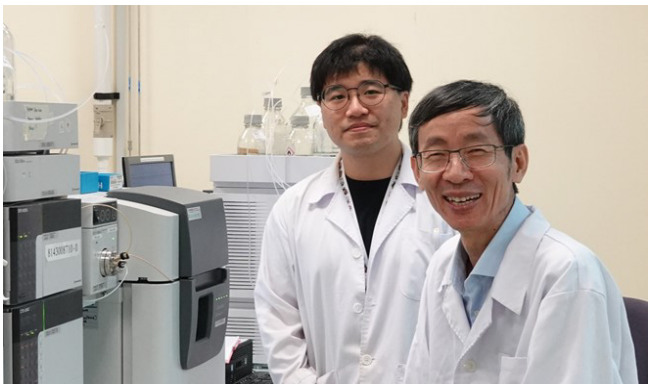
Ricco TINDJAU (left) is a Ph.D. student with the Department of Food Science and Technology. His interest lies in probiotic fermentation of foods, with a special interest in vitamins and biofortication of food.



Reference

[1] Tindjau R; Chua JY; Liu SQ*, "Growth and metabolic behavior of probiotic Bifidobacterium longum subsp. longum in minimally supplemented soy (tofu) whey" Future Foods Volume: 8 DOI: 10.1016/j.fufo.2023.100272 Published: 2023.

From a single cell to a full developed human

Discover how gene regulation shapes us from the womb and holds keys to treating genetic diseases

Figure 1: Bosma arhinia microphthalmia syndrome (BAMS) (left) and facioscapulohumeral dystrophy (FSHD) (right) are both caused by mutations in SMCHD1. One affects craniofacial development, the other causes muscle weakening.

Introduction

All of us start from a single cell, a fertilised egg, and undergo a remarkable transformation developing into complex human beings with many different cell and tissue types. While every cell within us share identical DNA sequences, they exhibit diverse forms and functions. This amazing diversity comes from each cell selectively turning on a specific set of genes while keeping others turned off.

Our laboratory has a long-standing interest in gene regulation, specifically during the early stages of development, known as embryogenesis. How do cells know which genes to turn on and when? How do they ensure that unwanted genes are kept in the off state? We study epigenetic repression, the process by which unwanted genes are turned off.

An epigenetic repressor for forming the nose

Our interest started from studying individuals born with developmental abnormalities. Bosma arhinia microphthalmia syndrome (BAMS) is an extremely rare condition characterised by the absence of a nose at birth, and in some cases, accompanied by eye and reproductive defects. Despite being documented for over three decades, its genetic basis remains unknown. Through global collaboration, we collected samples from affected individuals and conducted genome sequencing. Interestingly, all the patients had mutations in a single gene SMCHD1 [1].

SMCHD1 functions as an epigenetic repressor which is responsible for keeping certain parts of the genome inactive at all times. It acts like a guard to keep certain areas “off-limits”, so gene expression occurs only when necessary. Curiously, mutations in SMCHD1 were previously linked to a different disorder, facioscapulohumeral dystrophy (FSHD), a muscular dystrophy affecting up to 1 in 8,000 individuals. It is puzzling how mutations in the same gene lead to two different diseases, affecting different tissues with varying onset timings.

Our experiments with purified proteins and embryonic models suggest that FSHD mutations deactivate SMCHD1 function, preventing it from repressing the expression of a gene detrimental to muscle health. Conversely, BAMS-associated mutations appear to enhance SMCHD1 activity excessively, suppressing genes required for nasal formation.

Beyond explaining the mechanisms of how a nose is formed, our findings offer insights into potential treatments for FSHD. The race for a cure for FSHD is underway, with notable figures like Chip WILSON, founder of Lululemon and an FHSD patient, investing $100 million towards eradicating the disease by 2027. Finding ways to promote the activity of SMCHD1 could be key in this pursuit.

Mother’s genes control baby’s development

While studying embryogenesis,

we observed early and abundant expression of SMCHD1. When an egg is first fertilised, it does not make any of its own gene products. It is entirely dependent on the RNA and proteins the mother deposits in the egg during oogenesis. To study the function of SMCHD1 in embryogenesis, we turned to zebrafish as a model organism because it shares many developmental processes with humans and their abundant embryos facilitate research.

In zebrafish embryos, SMCHD1 is most abundant at the one-cell stage, meaning that the mother has loaded a lot of SMCHD1 into the egg. Through a series of genetic experiments, we discovered that maternal SMCHD1 is responsible for the correct expression of a set of crucial patterning genes known as the HOX genes in the embryo [2]. HOX genes control the identity of body parts in the developing embryo. In zebrafish that lost SMCHD1, many HOX genes are turned on prematurely, and over a broader region than usual. As a result, these zebrafish grew up having fewer bones in their vertebrae.

The most surprising finding we made was that the effect was solely attributed to the SMCHD1 provided into the egg by the mother. Zebrafish offspring from mothers with reduced SMCHD1 displayed abnormal vertebrae, even if they are genetically normal and can produce their own SMCHD1. This is not just a strange phenomenon in fish. An independent study using murine models showed a similar effect in mammals. Together, this suggests that SMCHD1 from the mother puts an epigenetic mark on the genome of the egg, instructions of some sort, to ensure correct expression of HOX genes during the offspring's development.

Figure 2: The mother puts in instructions onto the baby's DNA for turning on specific genes at a specific time. This ensures the proper development of the baby in the womb. [Image generated using an artificial intelligence tool]

The results of the study could change the way genetic diseases are interpreted. Diseases have always been thought to be caused by the patient's own genes. However, our research hints that some birth defects might stem from the mother's genetic mutations. This calls for the examination of the genetic make-up of the parents, especially in cases where genetic analysis of the patient comes up negative.

The next steps

From investigating nose formation to delving into embryonic patterning, our work with SMCHD1 is multifaceted. We continue our journey with this protein, moving on to study its operating mechanisms. By gaining an in-depth understanding of how it works, we aim to design therapeutic strategies for individuals living with FSHD. As we unravel the mysteries of gene regulation, we are reminded of the intricate choreography that turns a single cell into an entire human being – a process that is as complex as it is miraculous.

For more details, please visit: https://www.dbs.nus.edu.sg/staffs/xue-shifeng/

XUE Shifeng is an Assistant Professor with the Department of Biological Sciences, NUS. She obtained her Ph.D. in developmental biology from University of California, San Francisco and did postdoctoral training at A*STAR, Singapore, in human genetics. In recognition of her contributions, she was honoured with the Young Scientist Award in 2018 and the L'Oreal-UNESCO For Women in Science Singapore Award in 2023. Her laboratory studies epigenetic regulation in development and disease, with a focus on epigenetic regulators in rare genetic diseases.

References

[1] Gordon CT, et. al., "De novo mutations in SMCHD1 cause Bosma arhinia microphthalmia syndrome and abrogate nasal development" Nauture Genetics Volume: 49 Issue: 2 Page: 249-255 DOI: 10.1038/ng.3765 Published: 2017.

[2] Xue SF, et. al., "HOX epimutations driven by maternal SMCHD1/LRIF1 haploinsufficiency trigger homeotic transformations in genetically wildtype offspring" Nature Communications Volume: 13 Issue: 1 DOI: 10.1038/s41467-022-31185-8 Published: 2022.

Unlocking the secrets of cellular mechanotransductions

Targeting the supramolecular linkages in force transmission

Introduction

Mechanotransduction, which refers to the ability of cells to sense and respond to mechanical cues in local environments around them, plays critical roles in regulating various cellular processes. When this system malfunctions, it can lead to many diseases. Cells sense tensile forces through force-dependent interactions between force-bearing molecules and signalling proteins. These interactions trigger a series of events inside the cell. The effectiveness of mechanotransduction depends on the duration of the supramolecular interactions and the force required for activating the sensing parts of the proteins (mechanosensory protein domains). These molecular interactions, called force-transmission supramolecular linkages, form the physical basis of mechanotransduction.

Knowing how these crucial molecular linkages respond to mechanical forces is essential for explaining how cells sense and respond to their physical environment. This understanding could help modulate the strength of cellular mechanotransduction by targeting these linkages. However, the advancement of such understanding has been hindered by the lack of suitable experimental techniques capable of measuring physiologically relevant force levels and durations, as well as the mechanical responses of force-bearing protein domains and protein-protein interfaces (PPIs). Additionally, there is limited research on the binding affinity and kinetics between force-bearing binding sites and signalling proteins. Theoretical models to explain the fundamental biophysical principles underlying mechanosensing and mechanotransduction are also lacking.

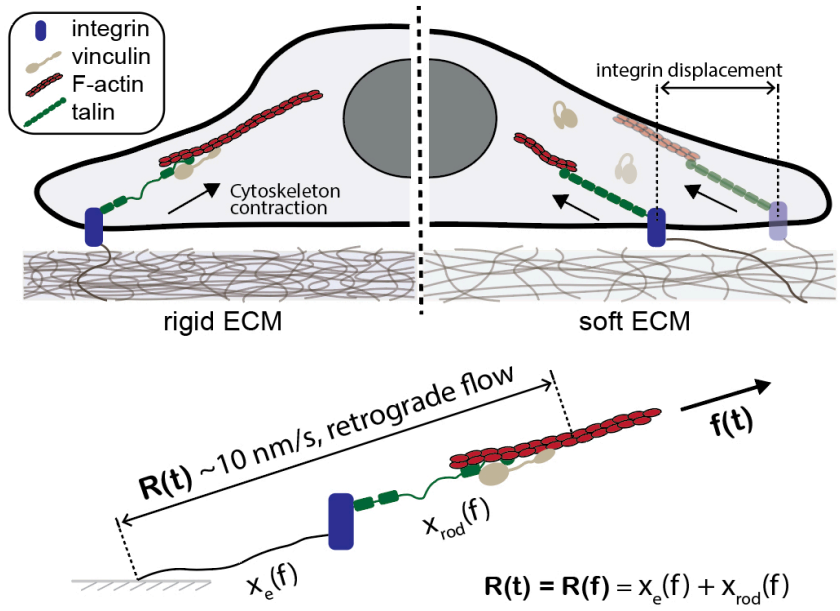


Figure 1: Membrane integrin connects the extracellular matrix (ECM) to an actin filament via talin and vinculin. Actin contraction pulls on this linkage, causing talin to unfold and switch binding partners from signaling proteins to vinculin, altering binding affinities.

Over the past decade, our laboratory has dedicated considerable effort on developing technology and theoretical models to help us and our peers in the field in measuring and comprehending the mechanical responses and interactions of these vital molecular linkages. Leveraging on the insights gained from these endeavours, we are now applying this knowledge to explore a new field called mechanopharmacology. This field involves adjusting the strength of mechanotransduction by targeting specific force-transmission supramolecular linkages.

The following perspective summarises the outcomes of our efforts, which have been detailed in several recent review articles [1-3], and outlines our future plans.

Mechanical linkages

Supramolecular linkages that transmit force are common in cells, coupling the actomyosin cytoskeleton to both the plasma and nuclear membranes via various receptors. These linkages organise cytoskeleton filaments into networks and are tensioned by actomyosin contractions and cell deformation. Besides providing structural support, they also function as sensors for mechanical forces. For example, cell adhesion to the extracellular matrix (ECM) involves integrin receptors connected to the cytoskeleton through mechanosensitive adapters like talin and vinculin, forming stable cell-ECM adhesions when the linkage is mechanically activated (see Figure 1). Similarly, cell-cell adhesion is mediated by cadherin receptors linked to the cytoskeleton through

proteins like catenins, resulting in strong adherence junctions when the linkages are mechanically activated. Nearly each component of these linkages is highly responsive to force and hence mechanosensitive.

Magnitude of the applied force

A significant advancement from our laboratory is the development of a highly stable tool called a magnetic tweezer (see Figure 2A). It is capable of applying a range of forces to individual protein domains or supramolecular linkages, while tracking their mechanical behaviour over long periods of time. Our research shows that most mechanosensitive protein domains destabilise when pushed or pulled with small forces ranging from a few to tens of piconewtons. However, the linkages, which comprise a tandem of structural protein domains can effectively buffer forces within the piconewton range over large distances. Our findings suggest that physiologically, individual force-transmission linkages can endure forces from a few to tens of piconewtons without breaking down. We have also developed a thin glass micropipette technology to control the position of ligand-coated microbeads and investigate the mechanical responses of ligand-binding receptors on cell membranes (see Figure 2B).

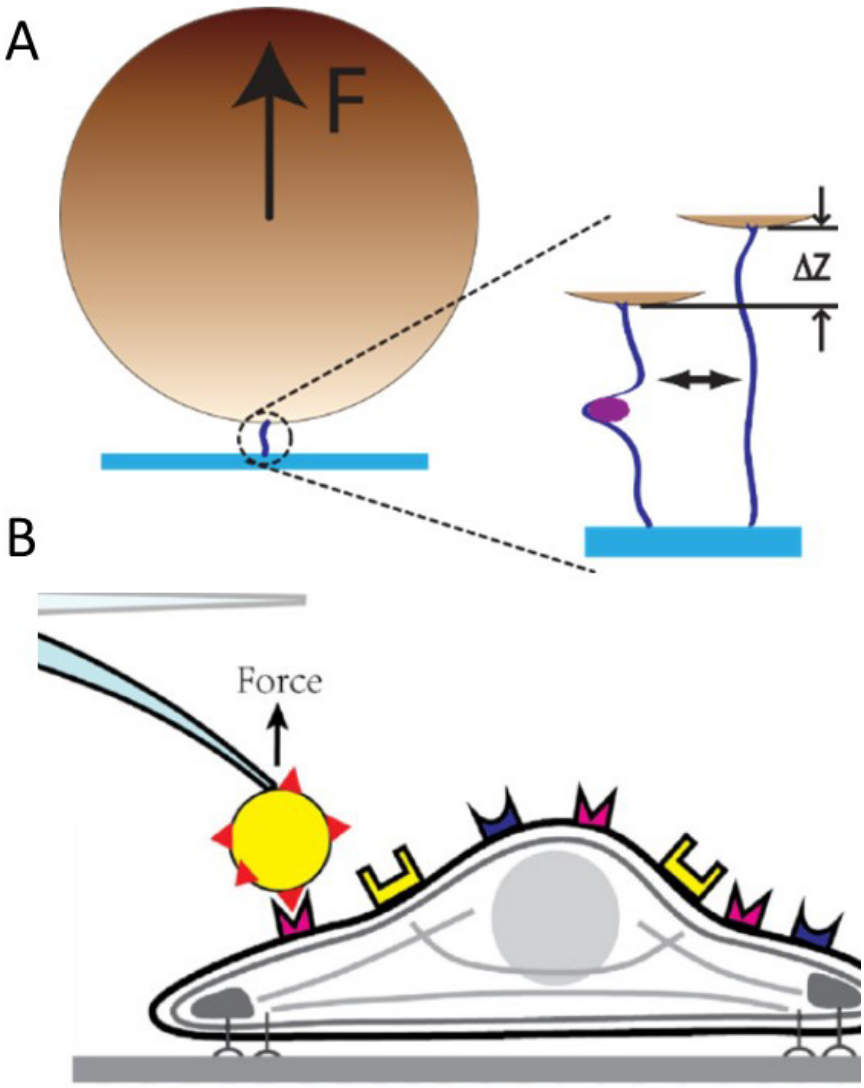


Figure 2: (A) Single mechanosensing biomolecule attached between a magnetic microbead and a glass surface, with force applied via the bead. (B) Microbead coated with ligand interacts with cell membrane receptors. Position and force applied by a thin elastic glass needle, monitoring cellular responses.

Duration of the applied force

It is not only proteins that get stretched, but many PPIs are also maintained under tension conditions. The duration of these PPIs is central for sustaining cellular processes; their disruption will halt mechanotransduction. Studying these single-molecule interactions under applied force is challenging because they can break, resulting in lost connections. We have addressed this by linking two protein domains with a sizable polypeptide chain, making it easier to reconnect them after they separate under applied force. With this method, we have analysed key PPIs and discovered that they can withstand stress from seconds to minutes, sustaining forces from a few to tens of piconewtons, indicative of their physiological resilience.

Force-dependent switching in binding partners

Protein domains can change shape under applied forces ranging from a few to tens of piconewtons. These domains often act as binding sites for signalling proteins and exhibit distinct interactions depending on their structural state. At forces below the unfolding threshold, the domains maintain their structures and bind with specific signalling proteins. Above this threshold, they change shape (unfold) and interact with different proteins. This means that the amount of force on these supramolecular linkages determines which proteins they interact with, reflecting the stiffness of the cell's environment. Through this approach, cells can detect and react to ECM rigidity and the tensile stress within cell-cell adhesion sites.

Talin's rod helical bundles, known to be rich in binding sites, can host over a dozen binding proteins. Typically, proteins bind to the folded bundles, but some, like vinculin and protein kinase A (PKA), attach exclusively to unfolded talin rods when these hidden binding sites are exposed. The mechanical stability of these rod domains determines the threshold for activation. Our research shows that

talin rod's domains unfold under small forces ranging from several to tens of piconewtons, which helps vinculin to bind strongly.

These studies help us understand how proteins sense and respond to forces, using force-transmitting linkages to detect the stiffness or flexibility of their surroundings..

Force-dependent binding constants

Biochemistry bridges the fields of biology and chemistry, exploring the molecules of life, including their formation, structure, functions and interactions. However, the biochemistry underlying mechanotransduction remains underexplored, largely because the involved molecules interact under mechanical stress – an experimental condition which is not typically considered in conventional biochemical research methods.

Our research has pioneered single-molecule assays that measure the force-dependent affinity between a stretched binding site and its partners in a liquid solution. We found that forces in the piconewton range can significantly alter binding affinities by several to tens of thousands of times, depending on the structural elasticity of the binding site pre- and post-binding. For example, the affinity of vinculin for talin can vary by thousands of times depending on the applied force. These findings illustrate the force-dependence of cellular interactions and are further elaborated in our recent review, where we discuss the associated theoretical biophysical principles.

Force-dependent rates

Our research shows that mechanosensory protein domains typically undergo force-induced structural transitions within a few to tens of piconewtons. However, the speed of these transitions vary considerably. Some domains unfold faster under higher forces, while others unfold slower. This also applies to force-bearing PPIs, with some breaking quickly, while others last a longer period of time.

The intricate kinetics of these transitions at the piconewton scale have intrigued theorists, but existing models largely lack in-depth physical explanations for these phenomena. We have pioneered a concept that highlights the significant influence of biomolecular entropic elasticity on transition rates at these forces. Utilising this insight, we have developed a theoretical framework that effectively correlates the force-dependent lifespans with the structural and elastic characteristics of biomolecular systems.

Future perspective – Mechanopharmacology

Controlling the duration and threshold force for mechanosensing proteins in force-transmission linkages can affect specific mechanotransduction activities. We envisage that these linkages could be promising drug targets to treat diseases linked to faulty mechanotransduction. They offer numerous drug-binding opportunities, including force bearing PPIs and mechanosensing domains. When targeting areas outside the cells, potential drugs could take the form of small molecules, peptides and large antibodies. Those that target areas inside the cell can be membrane-permeable molecules and short peptides. Our upcoming research will focus on screening drugs that target these mechanosensitive sites and evaluate their impact on cellular functions. The outcomes could significantly advance the field of mechanopharmacology.

For more details, please visit: https://www.physics.nus.edu.sg/faculty/yan-jie/

YAN Jie is a Professor with the Department of Physics, NUS and a principal investigator at the Mechanobiology Institute. A single-molecule biophysicist, he earned his Ph.D. from the University of Illinois at Chicago in 2005. He leads research in single-molecule studies, focusing on the micromechanics of DNA and proteins. His primary objective is to unravel the ways in which force-bearing proteins within cells can detect and react to mechanical forces and how these proteins can be targeted pharmaceutically. He is a Fellow of the American Physical Society and a Singapore NRF Investigator (2016 cohort).

References

[1] Guo YY, et al., "Mechanotransduction through protein stretching" Current Opinion in Cell Biology Volume: 87 DOI: 10.1016/j.ceb.2024.102327 Article Number: 102327 Published: 2024.

[2] Wang YN, et al., "Force-Dependent Binding Constants" Biochemistry Volume: 58 Issue: 47 Page: 4696-4709 DOI: 10.1021/acs.biochem.9b00453 Published: 2019.

[3] Le SM, et al., "Mechanical regulation of tension-transmission supramolecular linkages" Current Opinion In Solid State and Materials Science Volume: 25 Issue: 1 DOI: 10.1016/j.cossms.2020.100895 Article Number: 100895 Published: 2021.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