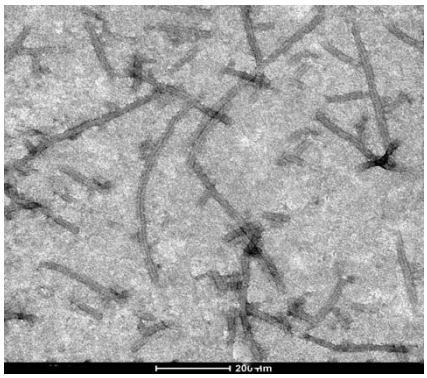
Plant virus-like particles as vehicles for therapeutic antibodies

-Anoushka Dasgupta

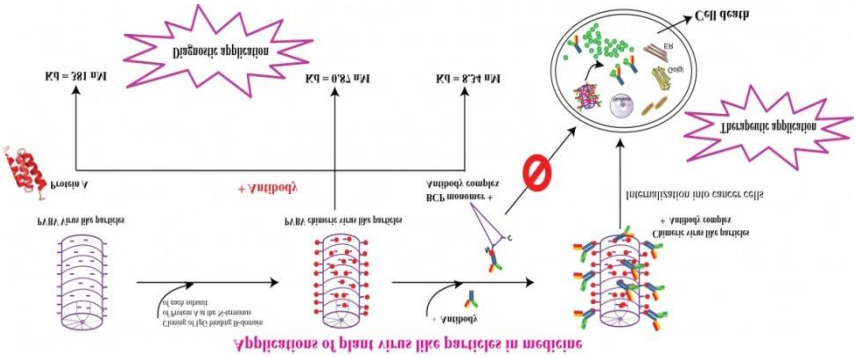
Monoclonal antibodies are those that originate from identical immune cells having a common origin. They are highly effective, non-toxic and can specifically target diseased cells, and are therefore used in immunotherapy to treat diseases such as psoriasis, cancer and autoimmune disorders. However, since antibodies are unable to cross the cell membrane, they have mainly been used against antigens present on the surface of cells.



Electron microscope image of chimeric virus-like particles of the Pepper vein banding virus (Credit: Pallavi Sabharwal)

Delivering such therapeutic antibodies into a cell to target antigens present inside requires a vehicle that can cross the cell membrane. Virus-Like Particles (VLPs) have this ability. VLPs have only the structural components of a virus but not the genetic material, which makes them non-infectious.

Now, researchers at the Indian Institute of Science (IISc) have developed a VLP of a plant virus called the *Pepper vein banding virus* (PVBV) to use as a possible vehicle to deliver antibodies into a cell. These VLPs can enter mammalian cells as well, despite being of plant origin.



Application of plant virus-like particles in medicine (Credit: Pallavi Sabharwal)

Previous research by the group has shown that plant VLPs that are spherical can be used to deliver antibodies. In a new study, published in the *Archives of Virology*, they

have shown this ability in a rod-shaped VLP. This rod-shaped VLP has an added advantage of a large aspect ratio, due to which its surface has a greater number of regions that antibodies can bind to. This allows the rod-shaped VLPs to carry more antibodies than spherical VLPs.

The researchers genetically engineered the PVBV VLPs by adding the antibody-binding B domain of a protein called Protein A from *Staphylococcus aureus* bacteria to an exposed region of the coat protein of the VLP. The resultant is called a chimeric VLP. This chimera, when exposed to the antibodies that need to be transported, can recognize and bind to them to form a stable complex.

“The interesting finding that we have shown is that these particles [only when] assembled can enter [the cell], and not their subunits,” says Prof HS Savithri, corresponding author of the new paper and NASI Senior Scientist at the Department of Biochemistry, IISc, Bengaluru. This, she says, is because there seems to be a scaffold formed by the assembled particles that the membrane receptors recognize, something that does not happen when they are individual protein subunits. These antibody-bearing chimeric VLPs can enter mammalian cells and deliver the antibody inside, where it can neutralize the target antigen.

The researchers also chemically combined fluorescent molecules to the surface of the VLPs, which allows them to track and therefore check if the VLPs are delivering the antibodies to the right location. “This is the important finding [of this research] – that we can make antibodies enter the cell and show that the antibodies delivered are functional,” says Savithri. This technique works if assembled viral structures are used.

The study is a proof of concept showing the advantages of using biodegradable, non-infectious and rod-shaped plant VLPs. Further research can be conducted on animal models to test the delivery of antibodies to specific cells, such as cancer cells. This can have immense therapeutic potential for treatment of diseases such as cancer and neurodegenerative disorders.

REFERENCE:

Pallavi Sabharwal, C. Sushmitha, C.K. Amritha, Usha Natraj, Mathur R.N. Murthy, and Handanahal S. Savithri. Development of pepper vein banding virus chimeric virus-like particles for potential diagnostic and therapeutic applications. *Archives of Virology*, 165: 1163-1176, 2020.

https://doi.org/10.1007/s00705-020-04581-y

A fluorescent way to evade UV radiation

Naturally occurring fluorescence has been observed in multiple organisms ranging from bacteria to birds. In macroscopic animals such as birds, fluorescence provides a visual communication signal. However, the functional significance of this phenomenon is unknown in most cases. In a recent study, researchers from the Department of Biochemistry have demonstrated the UV-protective role of fluorescence observed in a tardigrade isolated in the IISc campus.

Tardigrades (also called water bears or moss piglets) are microscopic animals (0.5 to 1 mm in length) with four pairs of legs. They are known for their ability to tolerate extreme physical stresses such as extreme temperature and pressure, ionising radiation, osmotic stress, and even the vacuum of space at low Earth orbit.

In the study, the researchers identified a new species of tardigrade belonging to the genus *Paramacrobiotus* within the IISc campus. This tardigrade can survive germicidal UV radiation. Suprisingly, these tardigrades use fluorescence as a mechanism to resist lethal ultraviolet radiation – they use a fluorescent shield that absorbs the harmful UV radiation and emits harmless blue light as fluorescence. The team was also able to transfer this UV tolerance property to another tardigrade, *Hypsibius exemplaris* and to a nematode, *Caenorhabditis elegans*, which are otherwise sensitive to UV radiation.

Reference:

Suma HR, Prakash S, Eswarappa SM. Naturally occurring fluorescence protects the eutardigrade *Paramacrobiotus* sp. from ultraviolet radiation. ***Biol Lett***. 2020 Oct 16(10):20200391. doi: 10.1098/rsbl.2020.0391 (Altmetric score: 488)

Lab website:

https://sites.google.com/view/dr-sandeep-m-eswarappa/home

Avoiding aggression can lead to species divergence

Like humans, animals are constantly communicating with members of their own species, sometimes using elaborate colours and behaviours to attract mates or repel rivals. In two fan-throated lizards – *Sitana laticeps* and *Sarada darwini* – males court females and display aggression to rival males by rapidly flagging their dewlaps, fan-shaped appendages below their throats.

In a small grassland patch in Maharashtra, these species overlap with each other. Similarities in male signals, and the fact that females of both species look almost indistinguishable, makes it challenging for males of both species to distinguish between rivals and mates of their own and other species. This apparent confusion triggers aggression between males. Aggression between these species can be intense and can escalate to chasing and even physical combat, which is particularly dangerous for the smaller *Sitana laticeps*.

A new study from the Centre for Ecological Sciences (CES) finds that in order to reduce this costly aggression with *Sarada darwini*, males of the *Sitana laticeps* species have altered their sexual signals and learnt to distinguish between rivals of their own and other species. In fact, compared to areas where these species live apart, *Sitana laticeps* in areas that overlap with *Sarada darwini* have smaller dewlaps and lower intensity of courtship and aggressive displays. Interestingly, these shifts in sexual signals of *Sitana laticeps* make them look a little more like females, and therefore reduce aggressive attention from the larger *Sarada darwini*.

Overall, aggression between species can play an important role in shaping the evolution and diversification of animal communication.

REFERENCE:

Zambre AM, Khandekar A, Sanap R, O'Brien C, Snell-Rood EC, Thaker M. 2020 Asymmetric interspecific competition drives shifts in signalling traits in fan-throated lizards. Proc. R. Soc. B 287: 20202141. https://doi.org/10.1098/rspb.2020.2141

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Sarada darwini (Photo: Swapnil Pawar)

Meet the new species of burrowing frog from *Namma* Bengaluru

Amphibian discoveries in India have been on the rise in the recent past, but most of them have been in biodiversity hotspots or forested landscapes with green cover. While documenting amphibians in parts of Karnataka that fall under the Deccan Plateau, a multi-institutional team including researchers from IISc encountered a new species of burrowing frog on the outskirts of Bengaluru. The species was named *Sphaerotheca bengaluru*, in recognition of Bengaluru's reputation as India's Silicon Valley. The new species was described based on morphological and genetic differences with known species of burrowing frogs across South Asia.

The discovery of the new species from the periphery of Bengaluru city highlights the importance of non-forested landscapes. Historically, Bengaluru was known as the "garden city" for its lush green cover and a large number of freshwater bodies. With growing urbanisation, the green cover has diminished and water resources have become scarce for "ecological indicators" such as frogs.

The new species is currently found in the peri-urban zones of Bengaluru which is dominated by agroecosystems mixed with dry deciduous vegetation without permanent water resources. More detailed field studies are needed to understand its distribution range and its natural history.

REFERENCE:

Deepak, P., K.P. Dinesh, A. Ohler, K. Shanker, B.H. Channakeshavamurthy & J.S. Ashadevi (2020) A new species of *Sphaerotheca* Günther, 1859 (Anura: Dicroglossidae) from the degraded urban ecosystem of Bengaluru, Deccan Plateau, India. *Zootaxa* 4885 (3): 423-436.

https://www.mapress.com/j/zt/article/view/zootaxa.4885.3.6

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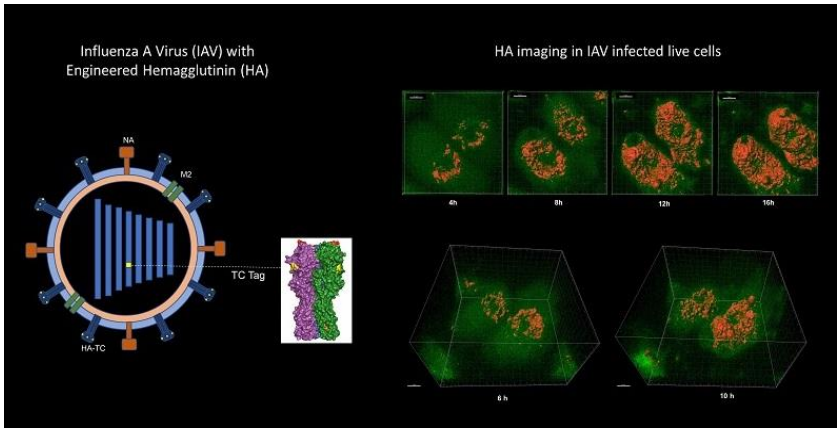
A novel method for live imaging of influenza infection

Samira Agnihotri



Influenza A viruses (IAV) are a well-known group of viruses, subtypes of which cause the “flu” in birds and some mammals, including humans.

Influenza hemagglutinin (HA) is a glycoprotein on the surface of these viruses that binds to the membranes of host cells to enable viral entry, and thus plays an important role in the infection process. Scientists have extensively studied this protein’s structure and synthesis. However, little is known about how HA moves through the network of organelles inside the host cell, and how it reaches the host cell membrane. Live imaging of the HA in IAV infected cells can enable such studies. An international collaboration of researchers, including Shashank Tripathi at the Centre for Infectious Diseases Research (CIDR), has developed a new method to enable the visualisation of the HA protein in infected cells.

This technique involves engineering a recombinant virus (called HA-TC PR8 IAV), which has a tetra cysteine (TC) tag that emits fluorescence in the presence of biarsenic dyes, and can be rapidly detected.

This method can be used to study the IAV infection process even after viral fusion with the host cell membrane, and could aid the discovery of antiviral drugs.

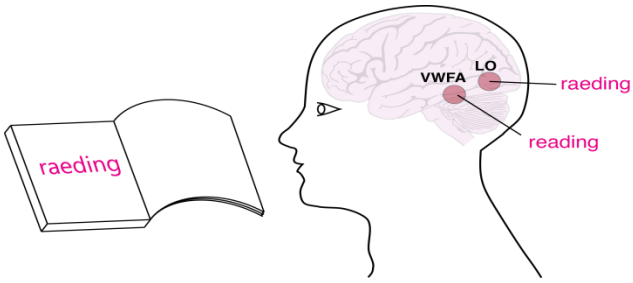
Reference:

dos Anjos Borges, L.G.; Pisanelli, G.; Khatun, O.; García-Sastre, A.; Tripathi, S. Live Visualization of Hemagglutinin Dynamics during Infection by Using a Novel Reporter Influenza A Virus. *Viruses* **2020**, *12*, 687. https://doi.org/10.3390/v12060687

Lab website: http://cidr.iisc.ac.in/shashank/

If you cna raed tish your vsion si aewosme

We have no trouble reading wrods veen fi htey aer jubmeld. How does our brain do this so effortlessly? Since reading a word involves both visual processing of letter and word shapes, as well as processing the associated sounds, phonemes, syllables and its meaning, it has been difficult to tease apart which of these processes explain our ability to read jumbled words.



Our goal was to investigate the extent to which purely visual processing can explain jumbled word reading. Our specific hypothesis was that when we see a string of letters, it automatically activates an efficient visual representation that is then matched to stored words.

To measure purely visual processing, we devised a model in which neurons respond to single letter shapes, and obtained their responses to longer strings by adding their response to single letters. We then asked whether the timetaken by humans to solve a particular jumbled string can be understood in terms of the similarity between the activity of neurons to the jumbled string and the original word. To our surprise, we obtained an extremely good fit: this model explained how long humans take on jumbled word reading tasks without any additional terms related to the word sounds or meaning.

To identify which brain regions were involved in such tasks, we made subjects perform the same task inside an MRI scanner. We found that viewing a string activates a perceptual representation in the higher visual cortex, whereas subsequent comparisons to stored words are made in the visual word form area.

Our results explain how word reading occurs in the brain, by explaining jumbled word reading using simple visual rules, and by assigning specific functions to word processing regions in the brain. These findings could prove useful in diagnosing and treating disorders of reading. We speculate that at least a subset of people with reading disorders might have abnormal visual processing that we can index or diagnose with visual tasks such as those used in our study. Such disorders could potentially be treated by targeted training for visual processing improvements.

This study was funded by a Senior Fellowship awarded to SP Arunby the DBT/Wellcome Trust India Alliance, and by the DBT-IISc partnership programme.

Full citation :

Agrawal A, Hari KVS & Arun SP (2020) A compositional neural code in high-level visual cortex can explain jumbled word reading. eLife 9:e54846 https://elifesciences.org/articles/54846

Faculty website links
Prof. SP Arun : https://sites.google.com/site/visionlabiisc
Prof. KVS Hari : https://ece.iisc.ac.in/~hari/

Devil in the details: Altered diffusion linked to AD

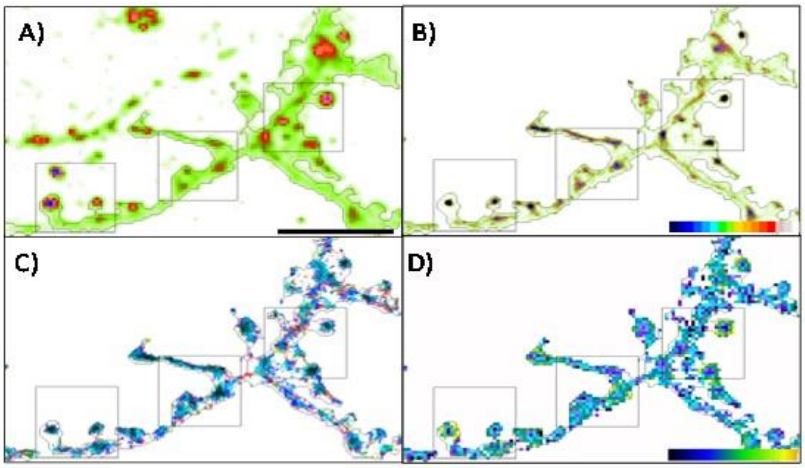
Synapses are connections that control information flow between neurons,and between neurons and non-neuronal cells in the brain and spinal cord. The molecular make-up of the synaptic membrane controls the efficiency of information transfer. In Alzheimer's disease (AD), the normal functioning of the synapses is disrupted, which speeds upthe subjects' cognitive decline. Studies have linked this declineto the clumping of a peptide known as amyloid beta, which is formed from the breakdown of a transmembrane molecule known as Amyloid Precursor Protein (APP). Although the biochemical pathways resulting in APP breakdown are well understood, how APP is organized into different neuronal compartments is not clear. This is partly because the nanoscale organization of APP in the neuronal compartments has not been studied in real time.

Using advanced imaging techniques that allow visualization of individual proteins, a research team led by Deepak Nair, Assistant Professorat the Centre for Neuroscience, IISc, has mapped out the organization of APP in different neuronal compartments. The team found that the APP molecules are packed into zones of high molecular density in neuronal processes. These molecules randomly "walk" in and out of nano-sized regulatory domains. The molecules are stalled only in these domains, while their movements elsewhere are unhindered, the researchers found.

The team then checked to see if this walk is altered in a variant of APP implicated in familial Alzheimer's disease. They found that this variant walked slower on the membrane, and was stuck in these regulatory domains for a longer time than normal APP molecules.

Working with computational biologists led by Suhita Nadkarni, Assistant Professor at IISER Pune, the researchers also created geometrical models of the synapse. Insights from these models showed that even minor alterations in individual APP molecules affect its movement, as well as the availability of APP molecules in a given area at a given time.

The study, therefore, highlights an important regulatorymechanism critical for understanding how changes in movement or aggregation of APP molecules can contribute to the onset of Alzheimer's disease. The next steps would be to validate this hypothesis and create smart probes that can alter the nanoscale behaviour of APP, which can influence the rate of amyloid beta production.



Lateral exchange and reversible immobilization of APP in live hippocampal neurons

REFERENCE:

Real-time nanoscale organization of amyloid precursor protein, S. Kedia, P. Ramakrishna, P. R. Netrakanti, M. Jose, J. Sibarita, S. Nadkarni and D. Nair, *Nanoscale*, 2020, 12, 8200. **https://doi.org/10.1039/D0NR00052C**
https://www.alzforum.org/papers/real-time-nanoscale-organization-amyloid-precursor-protein#comment-35441

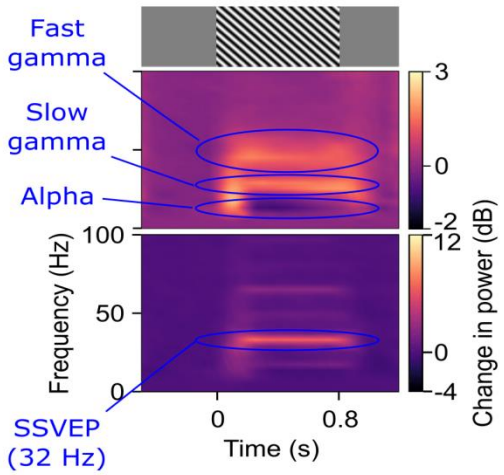
WEBSITE: http://www.cns.iisc.ac.in/home/people/deepak-nair/

Gamma oscillations in the elderly human brain weaken with age

-Priti Bangal

The activity of the human brain can be recorded as electrical signals. These include gamma oscillations which are examples of high frequency oscillations, with frequency ranging from 20-70 Hz. In areas of the brain that process vision, these rhythms are induced by specific images called gratings. Another type of rhythmic activity induced by flickering images of gratings are called Steady-State Visually Evoked Potentials or SSVEPs.

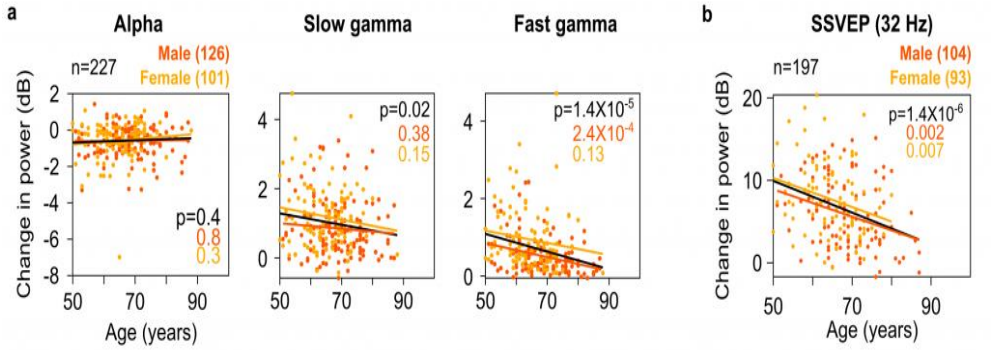
Now, researchers from the Centre for Neuroscience at IISc have shown that both visual gamma oscillations and SSVEPs weaken with age in healthy elderly humans.



Example of time-frequency change in power spectrograms, showing changes in alpha, slow gamma, fast gamma and SSVEP at 32 Hz, with respect to the power before start of the stimulus (baseline).An example stimulus (visual grating) is also shown and corresponds to the start (0 s) and stop (0.8 s) of its presence on the screen. For measuring gamma, we used stationary stimuli, and for measuring 32 Hz SSVEP, we used gratings that phase-reversed at 16 Hz. (Credit: Murty V P S Dinavahi)

During their study, the researchers presented different gratings to 227 healthy elderly participants (aged 50-88 years) on a computer screen, while they recorded brain electrical activity using electro-encephalography (EEG). EEG is a non-invasive procedure analogous to ECG and is performed using 64 electrodes spread across the scalp. They also collected data from 46 younger participants (aged 20-49 years) for comparison.

In their previous work, the authors had shown that there are two distinct gamma oscillations – fast (36-66 Hz) and slow (20-34 Hz) – in the occipital area (that processes visual stimuli), a finding that was novel to this field of research. In the current study, they have demonstrated that both these oscillations diminished with healthy aging, even though fast gamma showed a sharper decline in strength, as compared to slow gamma. Similar results were seen for SSVEPs at 32 Hz, but not in other oscillatory ranges such as alpha (8-12 Hz).



Scatter plots and regression fits for change in alpha/gamma/SSVEP power values relative to baseline across agefor elderly subjects (male: dark orange; female: light orange; both genders combined: black). (Credit: Murty V P S Dinavahi)

The authors believe that this work has implications in designing biomarkers for age-related disorders such as Alzheimer's disease and developing brain-computer interface applications for paralyzed elderly patients.

Reference:

Murty DVPS, Manikandan K, Kumar WS, Ramesh RG, Purokayastha S, Javali M, Rao NP, Ray S (2020) Gamma oscillations weaken with age in healthy elderly in human EEG. NeuroImage 215:116826. http://www.sciencedirect.com/science/article/pii/S105381192030313X

Lab website: http://www.cns.iisc.ac.in/sray/

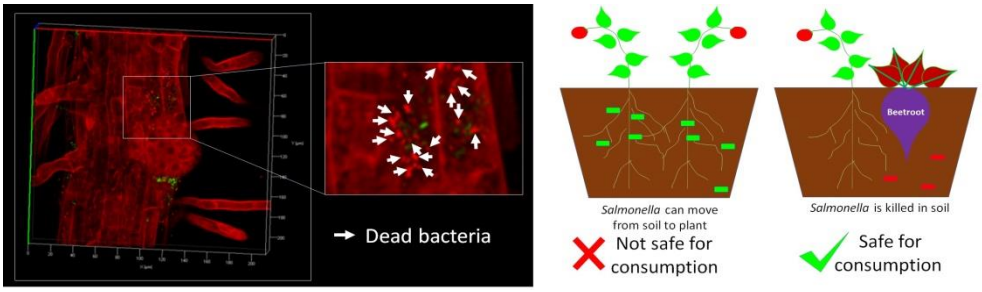
Beetroot as biocontrol in mixed-cropping systems

-Priti Bangal

Untreated sewage water and animal-based manure are a common cause of contamination in agricultural fields. Often, water is scarce, and manure cheap, which makes the use of these unavoidable. Through this route, food-borne pathogens like *Salmonella* bacteria may cause infections in humans.

To address this problem, researchers from the Indian Institute of Science (IISc) and University of Agricultural Sciences (GKVK), Bangalore, have developed a strategy to use beetroot as a cultivation partner with other vegetables to arrest the growth of *Salmonella* in them.

The team, led by Dipshikha Chakravortty in the Department of Microbiology and Cell Biology (MCB), found that some compounds released by beetroot plants had antimicrobial properties that reduce the growth of pathogens in food crops. The researchers tested this using different approaches: treating roots with *Salmonella* and testing the effects of beetroot extract on them, cultivating beetroot in *Salmonella*-treated soil, and growing tomatoes and beetroot together in *Salmonella*-treated soil.



Representative confocal image showing dead Salmonella *cells on root (Left). A cartoon showing that co-cultivation of beetroot with tomato can make the tomato safe for consumption (Right).*

The compound secreted by the beetroot plants is water soluble and can spread across the field through irrigation, without any extra effort. Beetroot can therefore be used a biocontrol, according to Kapudeep Karmakar, one of the researchers and a former PhD student at MCB.

Food crops that are consumed raw are at a greater risk of *Salmonella* infection. This method can hence prove important in cultivation of salad crops. It is also cost-effective and therefore of immediate importance to small-scale farmers.

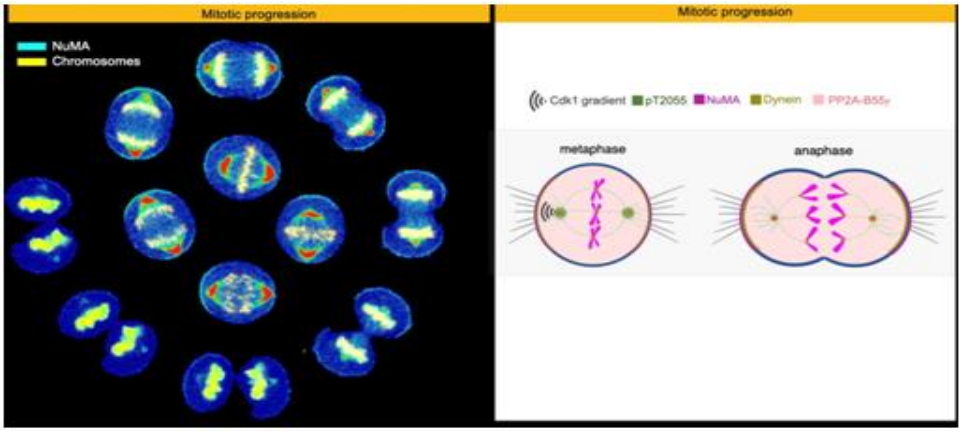
REFERENCE:

Karmakar, K., Krishna, S., Majumdar, S., Nath, U., Nataraj, K. N., Prakash, N. B., & Chakravortty, D. (2020). Co-cultivation of Beta vulgaris limits the pre-harvest colonization of foodborne pathogen (Salmonella spp.) on tomato. *International Journal of Food Microbiology*, 108768.

https://doi.org/10.1016/j.ijfoodmicro.2020.108768

A tug-of-war between two enzymes drives spindle forces in cell division

Rohini Murugan



When eukaryotic cells divide, thread-like structures called spindle fibres help to pull a copy of the replicated chromosomes into each of the daughter cells. The forces responsible for this pulling should be generated at the right time to ensure error-free cell division. In animal cells, a protein that plays an integral role in this process is NuMA (Nuclear Mitotic Apparatus protein).

One of the ways in which cells regulate the localisation and functions of such proteins is by adding or removing phosphate groups. When NuMA is dephosphorylated at anamino acid residue called Threonine 2055 (T2055), it is localised to the cell cortex (part of the membrane) where it helps anchor the motor protein, dynein, which is essential for generating the spindle forces. However, enzymes like Cdk1 counteract this process by phosphorylating NuMA at this amino acid residue, to block its cortical localisation.

Very little is known about the dynamics of these two processes. Researchers from IISc have now identified and characterised the subunit (B55γ) of an enzyme called PP2A, which is responsible for dephosphorylation of NuMA at T2055. They have also identified other residues in the NuMA protein sequence that are critical for dephosphorylation.

The researchers suggest that a tug-of-war between the two enzymes, Cdk1 and PP2A-B55γ, regulates the cortical levels of NuMA. Since low levels of B55γ are linked to

prostate cancer, future research will focus on unravelling the role of spindle formation in cancer progression.

Reference: Riya Keshri, Ashwathi Rajeevan, Sachin Kotak. “PP2A-B55γ counteracts Cdk1 and regulates proper spindle orientation through the cortical dynein adaptor NuMA”, *Journal of Cell Science* (2020) 133: jcs243857.

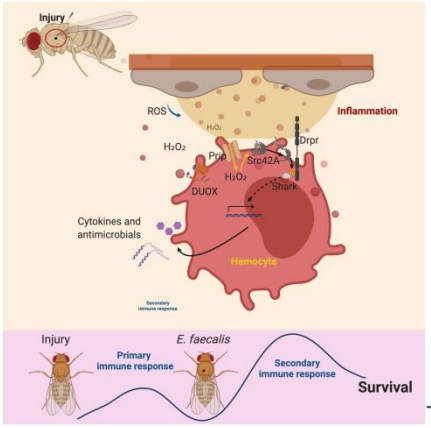
doi: 10.1242/jcs.243857

Lab website: https://kotakcellbiology.wixsite.com/spindlebehaviour

Unravelling signalling pathways in wound healing

Our cells sense invading microbes using specific signatures found in them called microbe-associated molecular patterns (MAMPs). Recognising these signatures activates signalling cascades that trigger specific immune responses. Our body can also detect signatures associated with tissue damage and wounds from its own cells. These signals are called damage-associated molecular patterns (DAMPs). Some example of DAMPs includes ATP, uric acid and heat-shock proteins. To study the signalling pathways linked to pathogen invasion and wound healing, researchers have been using the fruit fly as a model.

In a new study, researchers from IISc show that hydrogen peroxide produced from a wound activates specific signalling pathways in the fruit flies’ blood cells (also called hemocytes). Hydrogen peroxide acts as a DAMP signal to help home in hemocytesto the site of damage and activate wound-healing pathways. Hemocytes, in turn, help produce more hydrogen peroxide near the wound using an enzyme called DUOX.



The researchers also found that a water channel called aquaporin helps increase intracellular hydrogen peroxide in blood cells following an injury, which is critical for their activation. Another immune pathway called Toll pathway was found to be activated upon injury, which is protective for the flies from subsequent infection by bacteria. This points to a role that the injury has in training the immune response to fight a future potential pathogen.

The study was carried out by Sveta Chakrabarti, a India Alliance DBT/Wellcome Trust fellow and Sandhya Visweswariah, Professor at the Department of Molecular Reproduction, Development and Genetics.

REFERENCE: Sveta Chakrabarti, Sandhya S. Visweswariah, Intramacrophage ROS Primes the Innate Immune System via JAK/STAT and Toll Activation, *Cell Reports*, 33, 6, 108368 https://doi.org/10.1016/j.celrep.2020.108368

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Prof Sandhya Visweswariah's website: https://sites.google.com/view/sandhya-s-visweswariah/home