Cellular podiatry – understanding how cells form feet

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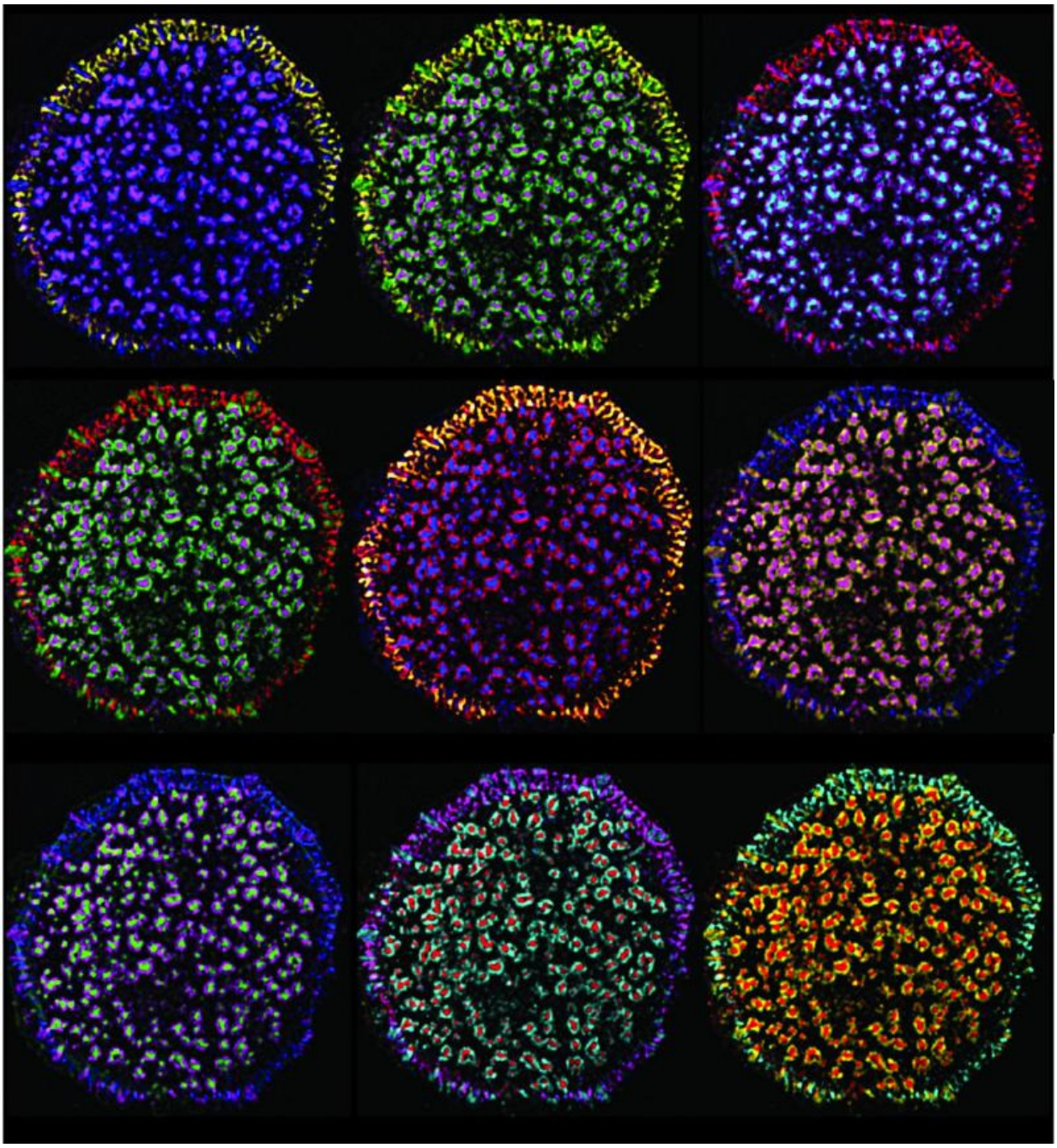

A podosome-forming immune cell viewed under a super resolution microscope in nine colour combinations. Podosomes, which are often described as cellular feet, are found underneath the cell surface and are seen here as dots within the circular cell boundary. Each podosome contains several proteins, including a core of actin, surrounded by a ring of vinculin. Fluorescent molecules are attached to these proteins and this allows them to be seen through the

microscope. Using computer software to change the colours of the light emitted by the fluorescent molecules, Ms Nisha Rafiq, a PhD candidate at the National University of Singapore (NUS), has created the nine colour combinations that artistically show podosomes in the cell. These images were created as part of a study led by Nisha, and Principal Investigator, Professor Alexander Bershadsky, from the Mechanobiology Institute, Singapore at NUS into how podosome formation is controlled by a protein known as Arf1. Credit: National University of Singapore

A study carried out by a team of researchers from the labs of Professor Alexander Bershadsky at the Mechanobiology Institute, Singapore at the National University of Singapore and Professor Gareth E Jones at King's College London has revealed that a protein known as Arf1 plays a role in podosome formation by regulating the assembly of myosin-II within the cytoskeleton. This study was published in the *Journal of Cell Biology* in December 2016.

Molecular players in podosome formation

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

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

often present (known as invadopodia) and these participate in the processes of cancer cell invasion and metastasis.

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

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

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

Following this line of investigation, the team went on to discover that removal of Arf1 from cells rendered them incapable of forming podosomes. In this case, Arf1 was controlling proteins involved in the

formation of the cytoskeleton. One protein found to be directly impacted by Arf1s presence in podosomes was myosin-II, a protein that confers contractility to the cytoskeleton. Importantly, myosin-II is not found in the cytoskeletal core of the podosome, however it is found at the periphery. When Arf1 was reduced, myosin-II increased, suggesting this molecular switch controls how much mysosin is present in the area, as too much would prevent podosome formation.

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

More information: Podosome assembly is controlled by the GTPase ARF1 and its nucleotide exchange factor ARNO, DOI: 10.1083/jcb.201605104

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