

Understanding development and disease: prostaglandins coordinate actin cytoskeletal remodelling

At the University of Iowa, **Dr Tina Tootle** leads her own laboratory focused on uncovering the actions of prostaglandins. Prostaglandins are lipids that play critical roles in human health and disease. Specifically, her research examines the roles of prostaglandins in regulating actin. As one of the most abundant and highly conserved proteins, failure in actin regulation has been shown to lead to developmental defects and diseases such as cancer and neurodegeneration. Hence, it is important to gain a better understanding of the interaction between prostaglandins and actin.

The actin cytoskeleton plays a vital role in the development of tissue homeostasis, cell division and migration as well as shaping the cell structure. As such, tight regulation of actin is required to ensure functioning and is mediated by actin-binding proteins. Though experimentation has identified mechanisms by which single actin-binding proteins are controlled, more comprehensive insight into how the activities of multiple actin-binding proteins are coordinately regulated is required to understand actin's influence on developmental and disease processes.

PROSTAGLANDINS REGULATE THE ACTIN CYTOSKELETON

Dr Tootle's research has identified prostaglandins as one such possible mechanism. These represent a group of lipid compounds that mediate a wide variety of biological processes, including female

reproduction, sleep, pain, immunity and cancer. Notably, the cyclooxygenase (COX) enzymes that produce prostaglandins are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin.

Prostaglandins can regulate the actin cytoskeleton in a wide variety of ways and have been shown to modulate actin and membrane interaction, cell migration as well as stem cell activation. Using *Drosophila melanogaster*, a fly species more commonly known as fruit fly, Dr Tootle has found that prostaglandins also regulate female fertility and various actin-dependent processes within *Drosophila* follicle or egg development. Examination of egg development in *Drosophila* also uncovered the COX-like enzyme, Pxt, and genetic disturbances of prostaglandin signalling, which have aided in examining prostaglandin function.

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EGG DEVELOPMENT SHEDS NEW LIGHT ON REGULATION MECHANISMS

Drosophila oogenesis – the maturation of female gametes – is a well-studied model to explore actin cytoskeletal regulation. It comprises 14 morphological stages of follicle development. During Stage 10, Pxt-driven prostaglandin signals initiate actin remodelling necessary for the final stages of development (see top image overleaf). The significance of these lipid compounds becomes apparent in mutants where prostaglandins are unavailable and hence follicle development fails and the female is sterile.

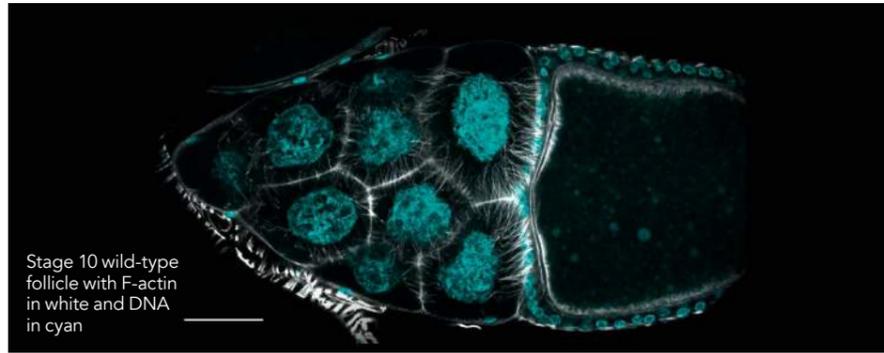
FASCIN – AN IMPORTANT DOWNSTREAM TARGET OF PROSTAGLANDINS

The Tootle Lab has also identified new factors mediating prostaglandin-dependent actin remodelling, including the actin-bundling protein Fascin. Specifically, Fascin acts as a downstream target of prostaglandins to facilitate late stage follicle morphogenesis. The loss or reduction of Fascin leads to actin defects. It further interacts with COX inhibitors, as well as the COX-like enzyme Pxt, to influence actin bundle formation, making it an important downstream target of prostaglandins. Surprisingly, recent collaborative studies by the Tootle and Parsons labs uncovered that Fascin also localises and functions in the nucleus and at the nuclear periphery. As Fascin has also been found to be upregulated in breast cancer, these new findings may be able to guide cancer drug therapy in the future.

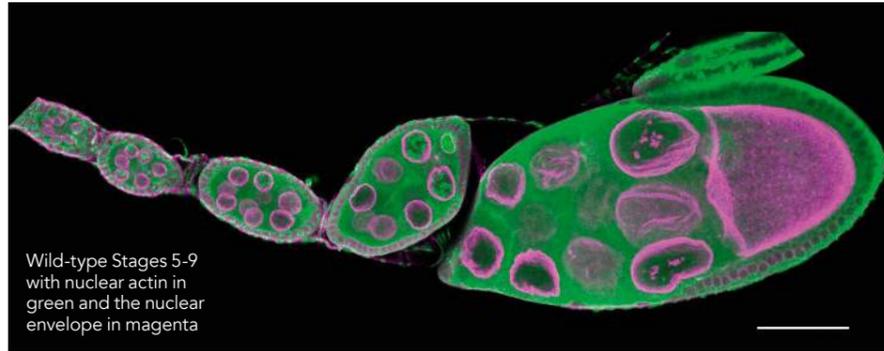
NUCLEAR ACTIN

More recently, Dr Tootle has begun to examine the role of actin in the cell nucleus. First found in the nucleus around 50 years ago, actin has been shown to play a crucial role in gene expression, chromatin remodelling and DNA damage repair mechanisms. Though little is known about the biochemistry of nuclear actin, it appears





Stage 10 wild-type follicle with F-actin in white and DNA in cyan



Wild-type Stages 5-9 with nuclear actin in green and the nuclear envelope in magenta

to act as monomers, short polymers, and rods. Actin rods are structures that have been observed when cells undergo stress, such as heat shock or starvation. These rods have also been observed in patients with actin myopathies, as well as neurodegenerative diseases including Alzheimer's, Huntington's and Parkinson's disease. Indeed, if not removed, actin rods exhibit a toxic function to the cell.

USING DROSOPHILA TO EXAMINE NUCLEAR ACTIN

Dr Tootle has begun to examine nuclear actin during *Drosophila* egg development. Employing immunofluorescent imaging techniques, she identified actin in the nucleus of specific cell types during early stages of oogenesis (germarium-Stage 9; see bottom image). She also found that germline expression of GFP-actin could induce nuclear actin rod formation during these same stages, resulting in a reduction of female fertility and even sterility. Overexpression of Fascin was

found to increase GFP-actin rod formation. Loss of Fascin, on the other hand, results in low levels of nuclear actin. Hence Fascin may have an effect on both the level and structure of nuclear actin. Recent unpublished studies in the Tootle Lab implicate prostaglandins in regulating Fascin to control nuclear actin. Together these findings highlight the role of nuclear actin regulation during oogenesis in *Drosophila* and further point toward an important function of nuclear actin in development. Actin cytoskeletal remodelling and nuclear actin are important factors for normal development and a variety of diseases. The research in the Tootle Lab aims to further explore contributing signalling connections between actin and prostaglandins. Using *Drosophila* genetics, cell biology, imaging methods and biochemical tools, Dr Tootle plans to offer more insights on this topic in the future. The results are likely to benefit cancer and neurodegenerative research and offer new paths towards improvements in therapeutic methods.

The Tootle Lab's work will benefit cancer and neurodegenerative research, offering new paths towards improvements in therapeutic methods

Q&A

Why is *Drosophila* a good model system for your research?

To put it simply, *Drosophila* or fruit flies can be thought of as little people with wings.

Fruit flies are a robust genetic model system that can be used to understand human health and disease. To date, ~70% of the genes associated with human diseases are found in the fruit fly. What makes flies really great is that they have less genetic redundancy, which means that while a human, or even a mouse, has multiple copies of a particular factor, such as COX enzymes or actin-binding proteins, a fly is likely to have only one of each. Additionally, because flies have been used for biological research for over 100 years, the system has incredible tools to manipulate practically every single gene in the fly; in my lab alone, there are over 1000 different fly stocks carrying different mutations or genetic tools. Putting these two facts together, it means we can rapidly determine every actin-binding protein regulated by prostaglandins, and move on to the more interesting questions of figuring out how. Additionally, flies are small, inexpensive, and reproduce rapidly, and few people have ethical concerns about using fruit flies for biomedical research.

In my lab, we use oogenesis as our primary model tissue. The fly ovary is incredibly organised and is made up of chains of sequentially developing follicles or eggs. This means we can see the whole process of follicle development many times over from a single fly. Additionally, one of the cell types within the follicle that we study is very large and allows us to visualise the actin cytoskeleton in great detail while actin is being dynamically rearranged. Thus, fruit fly follicle development is an ideal system to figure out how prostaglandins regulate actin dynamics.

Is the role of actin the same in the human body as in *Drosophila*?

Yes, actin functions the same in people as it does in the fruit fly. Actin is the most conserved protein across all organisms. It is ~90% identical from yeast to humans.

This level of conservation indicates that actin function is critical for life. Indeed, actin regulates cell division and cell shape in every organism examined. While it is clear that actin's cytoskeletal functions are the same, it remains to be established whether this is true for its nuclear functions. It seems likely that nuclear actin roles are also highly conserved. Supporting this idea, the bacterial actin-like protein controls the organisation of the DNA. In human cells, nuclear actin is also known to regulate DNA organisation. Additionally, nuclear actin rods form in slime mould, fruit flies, and humans in response to cellular stress.

Why did you choose to study prostaglandins?

Aspirin is the oldest commercial drug. It was marketed by Bayer in 1899 and acts by inhibiting the COX enzymes that produce prostaglandins. Thus, aspirin blocks prostaglandin production and signalling. Aspirin can relieve pain and inflammation, low doses promote heart health while high doses can cause damage to the heart and can cause reversible infertility in women, low doses may reduce the risk of cancer, and long term use or high doses can cause severe bleeding disorders. Therefore, aspirin and other NSAIDs can be thought of as sledge hammers: to design more targeted therapies it is essential to identify the downstream targets that actually mediate the cellular outcomes of prostaglandins. It is this area that my lab has focused its efforts on.

By taking advantage of the robust genetic tools for fruit flies, we have identified a number of new downstream targets of prostaglandins, including Fascin. Importantly, all of the identified targets are also implicated in cancer development, progression, and/or metastasis. As high levels of prostaglandins are involved in cancer progression and patient outcomes, we believe the same prostaglandin signalling pathways regulating Fascin during fruit fly follicle development are utilised to regulate Fascin in cancer.

Actin rods are formed in response to stressors. Is there a connection to ageing given their prevalence in neurodegenerative disease?

I think it is likely that nuclear actin, including nuclear actin rods, contribute to ageing. During ageing, cells are exposed to an increasing number of factors that induce cellular stress. Such stress may cause nuclear actin rod formation. It is important to note that nuclear actin rods are a cell survival mechanism. They form to stop certain processes in the cell to give the cell time to deal with and recover from the stress. It is only when the actin rods remain that their formation is toxic to the cell. I believe that nuclear actin, including actin rods, are likely to play roles in most cells in our bodies – contributing to tissue homeostasis or repair. Thus, like all cellular processes, things get worse with age, and the likelihood of stable and toxic nuclear actin rods increases. Supporting this idea, nuclear actin rods are observed at increased frequencies in older human brain tissue, and we see more rods in the follicles from older fruit flies.

Are there other stages during the *Drosophila* life cycle which you may wish to explore in the future and if so, why?

There are many interesting questions about prostaglandins, actin cytoskeletal regulation, and nuclear actin that can be examined using tissues other than oogenesis. Given the relationship of nuclear actin to neurodegeneration, we are very interested in expanding our studies to the fruit fly brain – during both development and ageing. Interestingly, prostaglandin signalling is upregulated in the brains of patients with neurodegenerative diseases. We are also interested in how traumatic brain injury impacts both prostaglandin signalling and nuclear actin. Another tissue where prostaglandins are likely playing critical roles is within the intestine or midgut of the fruit fly. The fly midgut is strikingly similar to the human intestine: the pathways regulating its stem cells and response to damage are the same. As prostaglandins are strongly implicated in colon cancer, we hope to use the simple fruit fly to uncover how misregulated prostaglandin signalling drives tumour development and progression.

Detail

RESEARCH OBJECTIVES

Dr Tootle's research focuses on understanding the roles of prostaglandins in relation to actin. Her previous studies have uncovered an exciting area of research and she is currently interested in studying the relatively unknown area of nuclear actin, particularly in terms of its structure and function.

FUNDING

- National Institutes of Health (NIH)
- National Science Foundation (NSF)

COLLABORATORS

- Maddy Parsons (King's College London)
- Kris DeMali (University of Iowa)
- Anna Allen (Howard University)

BIO

As a volunteer at the National Institute for Mental Health during high school, Dr Tootle was instantly drawn to science. She studied Microbiology at the University of Maryland and received her PhD in Biology from the Massachusetts Institute of Technology. Her interest in prostaglandin action using *Drosophila* developed during her postdoctorate at the Carnegie Institution for Science.

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