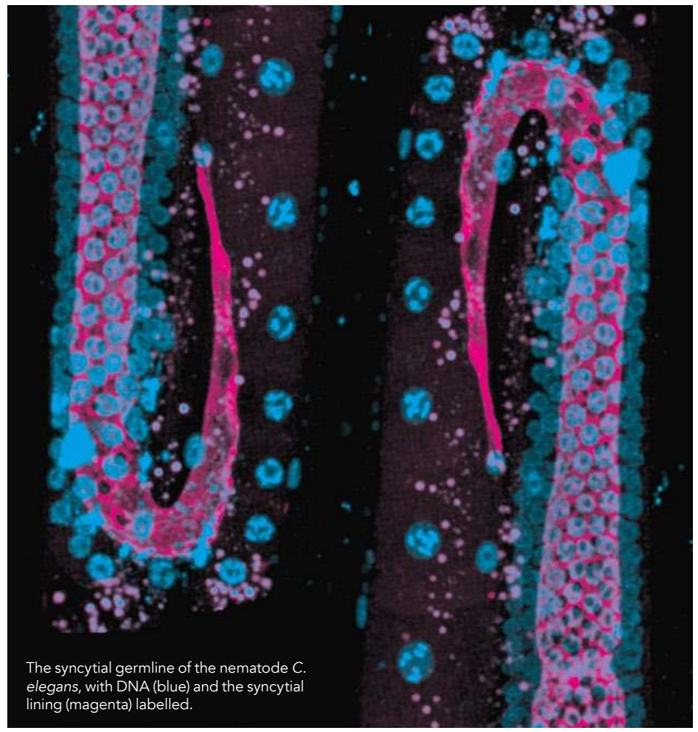
Building interdisciplinary bridges to study intercellular bridges

***Dr Amy Shaub Maddox** from the University of North Carolina is leading a diverse team of researchers to uncover the properties of intercellular bridges within multinucleated cells. Known as syncytia, cells with interconnected nuclei can be found in model organisms such as Drosophila and Caenorhabditis elegans as well as more complex organisms, including humans. Dr Maddox has also placed significant emphasis on outreach to a variety of audiences throughout the course of this research. The imaginative techniques the team is using are sure to inspire successful collaborations within the university community and beyond.*

The building block of all life is the cell. The architecture of a generic animal cell is well understood: a single nucleus resides in the cytoplasm, surrounded by the cell membrane. However, not all cells are generic. Both simple and complex animals in fact have some specialised cells with more than one nucleus. A cell with multiple interconnected nuclei is known as a syncytium. One place in the body where syncytia are found is the tissues that generate sex cells (sperm and eggs). In these syncytia, nuclei are connected to a common cytoplasm via intercellular bridges. The work of Dr Maddox and her team of researchers at the University of North Carolina focuses on the composition, dynamics and regulation of these bridges.



The syncytial germline of the nematode *C. elegans*, with DNA (blue) and the syncytial lining (magenta) labelled.

SYNCYTIA: WHAT? WHERE? AND WHY?

To understand more about the work of Dr Maddox and her colleagues, it is necessary to understand more about the syncytium. Though syncytia are a little different from the commonly taught norm, they are present throughout the animal kingdom. From the fruit fly, *Drosophila*, to humans, many species feature syncytial cells in their germline. The germline of a multicellular organism is the population of its bodily cells which allows it to pass on genetic material via sexual reproduction.

Amy (left) in the microscope room with members of her lab.

The structure of syncytia, including the properties of the intercellular bridges, has important consequences for fertility. Intercellular bridges allow for communication and coordination among the nuclei that reside there together, incompletely partitioned like horses in barn stalls. For example, in various types of syncytia, cytoplasm must flow out of some stalls and into others to enlarge them. When the oocytes are fully enlarged, the bridges collapse, thus achieving cellularisation. If the bridges collapse prematurely, the oocytes do not enlarge properly, and are not viable. Thus, these intercellular bridges must be stable. However, Dr Maddox's lab recently found that in the gonad of the model organism *Caenorhabditis elegans*, intercellular bridges are surprisingly dynamic throughout the germline, enlarging and contracting multiple times over their lifetime.

How syncytia form and are maintained remains poorly understood, however, research by Dr Maddox and others has begun to unravel some of the mysteries. For example, some of her earlier work identified a protein called *anillin-2*, which promotes the integrity of syncytia and endows the intercellular bridges with elasticity and stability. Dr Maddox's lab has now discovered another pair of proteins that regulate the stability of intercellular bridges. Interestingly, one is implicated in a human disease of brain vasculature, so their discoveries of its mode of action may help our understanding of that condition.

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STUDYING SYNCYTIA

The model organism for this study, *Caenorhabditis elegans,* is a tiny nematode worm with a history of providing answers to some of the big questions in modern science. In fact, work with *C. elegans* has garnered three different Nobel Prizes. Many characteristics of this worm make it a powerful organism for syncytial studies; not least the well understood architecture of its simple body plan and invariant lineage of its cells. Similarly to other model organisms such as the fruit fly, genomic modifications of *C. elegans* can be conducted with relative ease.

By manipulating the genome of *C. elegans* to introduce fluorescent protein tags, the team is using quantitative microscopy to gain unprecedented insight into the make-up of intercellular bridges. Quantitative microscopy allows for automated analysis of two- or three-dimensional images, meaning vast amounts of data can be analysed much more quickly than via manual measurement. Quantitative analysis is powerfully combined with high-resolution light microscopy techniques housed in the Maddox lab and within her department's shared equipment facility.

COLLABORATION ACROSS THE CURRICULUM

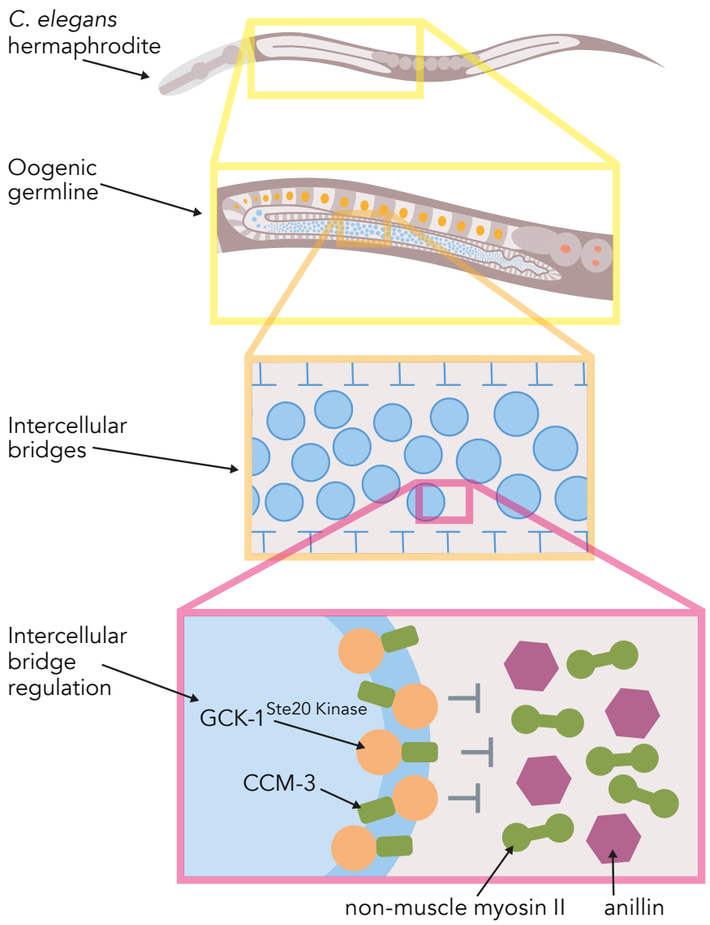
The team of researchers led by Dr Maddox share diverse geographical and educational backgrounds and the scope of the project allows for the training and mentoring of individuals at several stages of their education,

from undergraduate students to post-doctoral fellows. Amongst the lab members involved in the "hands on" research are Daniel B. Cortes, Kathryn Rehain-Bell and Michael Werner. Dr Maddox benefitted from the fact that the other researchers involved with this study are dedicated to and experienced with educational outreach. These activities ranged from interactive sessions with elementary school children to lab tours for prospective undergraduates.

Using the project to engage multiple audiences was very important to Dr Maddox. Her outreach activities sought out different groups within the University of North Carolina as well as individuals and groups in the local community.

One of the groups Dr Maddox was keen to involve with this project was the local education sector. She approached this aim in two ways. Firstly, each summer a teacher is invited to participate with the lab work for a 3-5 week internship. Dr Maddox hopes the experience will inspire these teachers to take their experience back to the classroom and relay their new knowledge and enthusiasm to their pupils. Secondly, Dr Maddox visits an elementary school to teach children about embryonic development. This classroom based project was developed according to established teaching goals and with the school's science specialist.

Dr Maddox also wanted to make the most of the already-collegial campus environment at the University of North Carolina by creating innovative opportunities for engagement. In order to improve interdisciplinary collaborations between the fields of biology and mathematics, Dr Maddox developed a module that allowed students from these courses to work alongside each other. In the module, the students experience novel methods of quantitative analysis and computer modelling using data they collect in the lab, studying intercellular bridges. One of the most original pieces of engagement proposed by Dr Maddox was interdisciplinary speed-dating. During these sessions, biologists briefly present projects to mathematicians,



Schematics of progressively magnified views of intercellular bridges (blue circles) in the *C. elegans* syncytial germline.

Our outreach activities will strengthen scientific research potential, not only during the funded period but for generations to come

physicists, computer scientists and statisticians interested in collaborations. Several successful partnerships have emerged from these events, and still more participants benefit just from the time spent building community.

Both the scientific and public engagement elements of this project provide numerous opportunities for future research. Dr Maddox is confident that the proposed outreach activities "will strengthen scientific research potential, not only during the funded period but for generations to come." Scientifically, there are still many more questions to be answered about the dynamics of intercellular bridges with such important consequences for so many species.

Behind the Bench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Research objectives
Dr Maddox and her team are attempting to achieve the following: understand intercellular bridge composition, dynamics and regulation, capture live images of cell dynamics using different C. elegans tissues, and also, conduct extensive outreach activities within the University of North Carolina and beyond.

Funding
- National Science Foundation (NSF)
- NIH's National Institutes of General Medical Sciences (NIGMS)

Collaborators
- Michael Werner, PhD
- Daniel B. Cortes, PhD
- Kathryn Rehain-Bell

The team acknowledges the valuable collaboration of Jian Liu (NHLBI), Francois Nedelec (EMBL), Adriana Dawes (Ohio State U), Wanda Strychalski (Case Western Reserve U), and many colleagues at UNC.

Bio
Team members have expertise in cell biology, high-resolution microscopy and computer-aided image analysis. Amy Maddox was trained in North Carolina and San Diego and has served as faculty in Montreal, Canada. Michael E. Werner was trained in genetics and developmental cell biology in Austria and Chicago. Daniel B. Cortes was trained in molecular and cell biology at the University of California at Davis. Kathryn Rehain-Bell was trained in evolutionary developmental biology at the College of William and Mary in Virginia.

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Q&A

What has been the most enjoyable and challenging aspect of your research?
It's funny that you ask it like that, but indeed the most challenging aspects are the most enjoyable ones. There are two classes of challenges: logistical and scientific. Logistically, it's challenging to keep the lab funded and keep the people happy, but successes in both these realms have been incredibly rewarding. Scientifically, our forays into mathematical biology have really stretched me, but we're making headway, mostly thanks to the fearlessness of trainees and generosity of collaborators. This diversification of our research program will doubtless propel us to new successes and enjoyment.

Were there any surprises for you due to working with such a diverse team?
I have actually been incredibly spoiled to recruit people not only with inner drive to study cell biology and development, but also with substantial training to tackle those questions. In fact, all three of my current post-graduate lab members were previously trained to use C. elegans, our model animal of choice. Since joining my lab, Michael, Daniel and Katie have diversified from each other by pursuing the knowledge, technologies and skills necessary to advance their projects and their independent careers. I admire their independence and inner drive, and am grateful to be part of their journeys!

What has been the best thing about working with other faculty members?
I really appreciate how my faculty colleagues provide a stimulating, multi-disciplinary environment, and express genuine interest in my scientific success. I am also grateful for their support of my sometimes-wacky ideas to connect people in new and different ways. They have been my "guinea pigs," attending scientific speed dating events and randomised lunch dates, and they report enjoying these activities! My most important colleague is my husband, who is a professor in the same department as I am. It is invaluable that we understand and can contribute to each other's careers.

Do you expect that future work of this nature will be conducted with C. elegans?
Yes, we certainly have not exhausted the potential for this model animal to reveal fundamental principles of syncytial biology. Even as more cell biologists are discovering the elegance and accessibility of this tiny worm's oogenic (egg-making) syncytial gonad, there are frontiers in the understanding the spermatogenic gonad, and in exploring the diversity of gonad architecture throughout nematode phylogeny and beyond.

How will a better understanding of intercellular bridge stability be useful for humans?
Since intercellular bridges are crucial for animal fertility, our discoveries will, in the long term, inform preventative or therapeutic medicine. Furthermore, findings of how multiple nuclei reside in one cell are relevant to other tissues (including heart muscle cells and certain fungi) with syncytial architecture. Importantly, "basic" research aimed at defining the workings of the natural world will always enrich humanity by deepening our understanding in general, and we can almost never predict how and when our findings will directly serve human health and society.