The marine sponge *Amphimedon queenslandica* has feelings too.

Do Sponges Get Nervous?

BY GEMMA RICHARDS

Despite lacking neurons, sponges are providing some intriguing insights into the evolutionary origins of nerve cells.

Nervous systems allow animals to sense what's going on around them and to respond to this sensory information in an appropriate manner. Without neural cells we wouldn't be able to see, hear, taste, touch or smell, and this would make complex behaviours and communication impossible.

The evolution of nervous systems is largely responsible for the success of the animal kingdom. With increasing sensory ability, animals have been able to colonise and exploit diverse habitats and to develop complex systems of behaviours.

However, there is one ancient group of animals that does not have nervous systems. This group branched off the animal tree before nervous systems evolved (in the same way that fish don't have legs because they branched off before legs evolved in higher vertebrates). This ancient group is the sponges, which have no nerves (and also no muscles) and live simple underwater lives attached to rocks or other hard surfaces.

In fact, for many years sponges were not even thought to be animals. Early scientists called them "Parazoa", which translates as "beside the animals".

We now know that sponges are indeed true members of the animal kingdom. They produce eggs and sperm, and their embryos undergo development into a larval stage that then swims around and explores the environment until it finds a suitable site to settle down and metamorphose into an adult sponge.

The swimming behaviours of sponge larvae demonstrate an ability to sense light and dark, and also to exercise choice over what kind of surface they settle upon. This choice may be based on "tasting" the molecules found on, or secreted by, these surfaces.

So, despite not having nerve cells, it is clear that sponge larvae can sense and respond to the environment around them.

We were intrigued as to whether the simple sensory abilities of sponge larvae bore any evolutionary relationship to the nervous systems of more complex animals. For several years our lab has been studying the marine sponge *Amphimedon queenslandica* and asking questions about its development, life history and ecology.

Recently the entire genome of *Amphimedon* was sequenced, meaning that we have access to the entire DNA instruction code for all the genes that belong to this sponge. With this information we can compare *Amphimedon* genes with the genomes of other animals.

When we find commonalities between *Amphimedo*n and other animals we can infer that these commonalities were inherited from their last common ancestor. For example, if both sponges and all other animals possess gene "X", we know that gene "X" must also have been a part of the genome of their last common ancestor, and thus the ancestor to all living animals. In such a way, using comparative genetics, we can examine genomic and morphological evolution within the animal kingdom.

Pioneering studies in comparative

genetics between higher animals, such as flies and mice, revealed that animal nervous systems are all basically built in the same way by the same genes. We decided to see if these same genes were also present in the *Amphimedon* genome and, if so, what they were being used for.

What we found was very surprising. Not only does the *Amphimedon* genome encode many genes that are used to create nerve cells in other animals, but these genes are all expressed during the development of the *Amphimedon* larva. Intriguingly we found that the genes were also all expressed in one particular larval cell type called a globular cell.

It was exciting that we found that the nerve genes were activated in this particular cell type for a several reasons.

First, the morphology and location of globular cells may indicate that they have a sensory capacity. They are found all around the outer surface of the larva when it is swimming around trying to find a settlement site, and one end of the cell sticks out into the external environment while the other end of the cell is inside the larva. The end that is outside the larva could be sensing chemicals (smell or taste) or surfaces (touch), and the cell may then transmit this information to the rest of the larva by releasing a chemical from its internal end.

Another interesting fact about these cells is that they were previously found to be a site of nitric oxide synthesis. We know this because the gene that makes the enzyme nitric oxide synthase, which makes nitric oxide, is expressed in the globular cells. Nitric oxide is a gaseous molecule that is often used as a signalling messenger in animals. It is made by cells in one part of an animal and then released internally to communicate a message to the rest of the animal. In many marine invertebrates the nitric oxide signalling system operates in the swimming larvae and is used to coordinate their metamorphosis from larval to adult form.

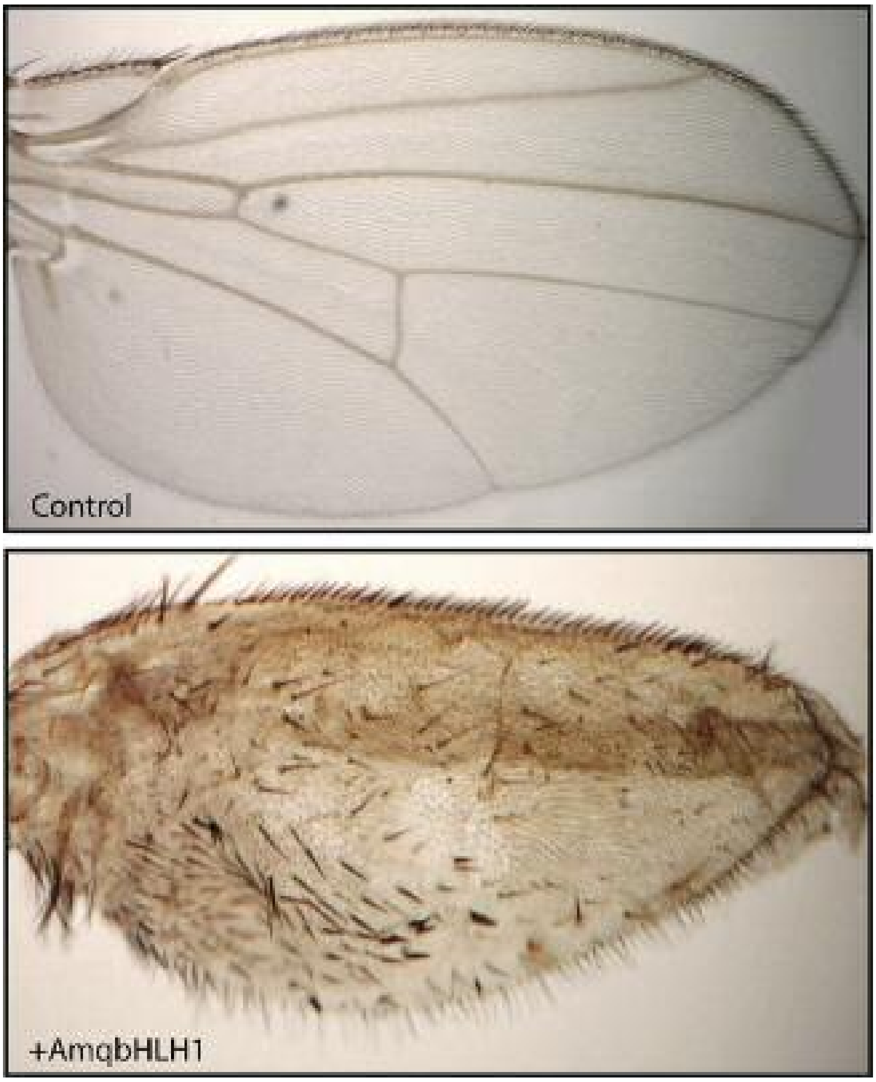
Finally, globular cells were already implicated in neural evolution due to a previous study investigating the evolution of the molecules that make up neural synapses (the highly specialised part of nerve cell membranes where communication between nerves takes place). This previous study found that many of the genes that make the structural proteins in synapses are also expressed in *Amphimedon* globular cells.

Together these studies provide several independent lines of evidence supporting a relationship between sponge globular cells and animal nerve cells. Both cell types share genes that create nerve cells, build nerve cell structures and synthesise chemical messenger molecules. A future challenge for us will be to identify the signals in the environment that globular cells respond to, and also how these signals are transmitted around the organism to affect its behaviour.

For the next stage of our research we focused on the functionality of one of the sponge genes that is related to important neural genes in other animals. This gene, *AmqbHLH1*, is in the same gene family as a large number of vertebrate genes that create nerve cells during animal development. While *AmqbHLH1* clearly does not make nerve cells in *Amphimedon*, we know that the relatives of this gene in other animals do have this ability.

To investigate *AmqbHLH1* further we isolated this gene from *Amphimedon* and then transferred it into two more complex animals: flies and frogs. First we injected *AmqbHLH1* into frog eggs and examined how that affected the development of the frogs' nervous systems. Remarkably, by injecting the sponge gene we caused the frogs to produce many more sensory nerve cells than normal – the sponge gene stimulated the creation of extra nerve cells!

We then did a similar experiment in flies, and again *AmqbHLH1* caused the flies to develop many extra sensory cells. In this case they developed extra sensory



While sponges do not have true nerve cells, the injection of the sponge gene *AmqbHLH1* into flies caused them to develop more sensory bristles on their wings.

bristles all over their wings. These were really striking results and demonstrated a clear relationship between the functionality of *AmqbHLH1* and its neural relatives in flies and frogs.

These findings have provided us with some stimulating evolutionary hypotheses. We know that sponges do not have true nerve cells, yet we have found that a range of genes that function in nerve cells are all localised to a particular sponge cell type, the globular cell. This common gene expression may indicate that nerve cells and globular cells have both descended from a single ancestral cell type that also had this gene expression.

We therefore are presented with two equally likely scenarios for when nerve cells first evolved in the animal kingdom.

In one scenario, nerve cells may have evolved before sponges branched off from the animal tree, but in sponges they degenerated into globular cells and lost a number of key neural features such as synapses and axons.

The other scenario would be that nerve cells evolved after sponges diverged, but that globular cells and nerve cells are both descendents of an ancient sensory "proto-neural" cell that subsequently evolved into globular cells in sponges and nerve cells in the rest of the animal kingdom.

By identifying common genes in sponges and other animals, and comparing where they are used and what they are used for, we can gain extraordinary insight into the genetic repertoire of the common ancestor to sponges and all animals, and thus into the earliest evolution of the animal kingdom.

In this study we have used comparative developmental genetics to identify a sponge cell type that may represent the evolutionary precursor to true nerve cells. Similar studies in our laboratory focus on the evolution of other key animal features such as immune systems, gametes and epithelial layers.

Despite appearing simple and unsophisticated, sponges and their genes are turning out to be full of surprises.

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