Your muscles and Fruit Fly stem cells

Dhananjay Chaturvedi*
Tata Institute of Fundamental Research, Bangalore
Email: dhananjayc@ncbs.res.in

For about 60 years we have known about stem cells that repair muscles in mammals. We were sure insect muscles don't use stem cells to repair themselves. But no one showed if and how insect muscles do repair themselves. This article discusses how the discovery of adult muscle stem cells in Fruit Flies was made.

We all get hurt from time to time. You might bruise a knee, break a bone or burn a finger. Our bodies possess the marvelous ability to largely repair these injuries. The ability to repair body tissues is not limited to just humans.

In multicellular life-forms, like ants and elephants (and us too), there are mechanisms by which one cell senses injury in its vicinity (***Doğaner B et al, Trends in Cell Biology 2016)***. Once this cell and other cells near the site of injury sense that there is a wound and it needs repair, they often proliferate to make the number of cells to replace damaged cells (***Duscher D et al,*** Gerontology 2016).

Not all cells can multiply if there is a cut. In adults, cells that can facilitate repair are called Adult Stem Cells (***Clevers H, Science 2015)***. When they divide, they give rise to two cell types. One cell takes the characteristics of the tissue which the stem cell came from. This change in characteristics is called differentiation. The other daughter cell remains a stem cell. This daughter stem cell can again divide to give rise to another differentiated cell and a stem cell. In this way, adult stem cells maintain and repair the tissue to which they belong.

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* Dr. Dhananjay Chaturvedi, Post Doctoral Fellow from National Center for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, is pursuing his research on "Muscle Repair through Adult Stem Cells in Drosophila." His popular science story entitled "Your Muscles and Fruit Fly Stem Cells" has been selected for AWSAR Award.

Cells within a body must coordinate growth and metabolism. Cells coordinate and communicate with each other at various distances through chemical signals. These signalling molecules can be small organic molecules like hormones or larger protein molecules. They can either be freely circulated in the body or attached to cell surfaces. Most often, a signalling molecule can bind another molecule on a recipient cell called a receptor. The combination of a signalling molecule bound to a receptor, triggers changes in the receptor-bearing cell. This signal—receptor combination is akin to a word that cells use to communicate in. If you injure yourself, say cut a finger, other cells in your finger sense the injury through such word(s). The signals trigger changes to repair the cut (***Oda Y et al, Journal of Steroid Biochemistry and Molecular Biology 2016)***.

A tissue most of us pay little or too much attention to is muscle. Muscles form 40-60% of our body weight. Skeletal muscles, the ones we use to move our bodies, are essential for quality of life. Imagine anything you did out of bed today. Now, picture doing these things without using muscles. Skeletal muscles are very different from other cell types in one important way. Each mature muscle cell, also called a muscle fibre, has thousands of nuclei. Each mature muscle cell comes to contain thousands of nuclei through the fusion of thousands of individual muscle cells with single nuclei. Several mature muscle cells together bundle up, like wires in a cable, to form what we all think of when we hear the word "muscle". These muscles contract and relax a lot. Movement damages muscles. The group of cells necessary for skeletal muscle repair is called 'satellite cells'.

Satellite cells are crucial to muscle repair. When muscles are damaged by severe motion or a physical injury, satellite cells divide over and over and their progeny fuse with muscle fibres (***Collins CA et al, Cell. 2005).*** If satellite cells are lost in adults, as can happen over time in certain genetic diseases, muscles lose mass and the ability to contract. Such patients can lose much of their ability to move objects or even their own body.

It turns out, these 'words' that cells communicate use with each other during repair, are also the same ones used during development from a zygote to an adult. Even more amazingly, these words are very similar between different species. In fact, we learnt the molecular nature of signalling mechanisms from the humble fruit fly.

Fruit flies are a great species to understand signalling mechanisms and the development of an organism. Of course, a fruit fly egg cannot develop exactly like and into a human being. But decades of studies have shown that the basic principles of tissue formation and function are very similar. We know now that the signalling and cellular mechanisms that breakdown to cause diseases like cancer in human beings, are very similar in fruit flies. In fact, many of them were first discovered in fruit flies.

So, to return to adult muscle stem cells or satellite cells: Through electron microscopy, scientists have seen this cell population in mouse muscle since 1961. These are muscle cells with single nuclei that are positioned right next to muscle fibres. If you take away satellite cells from mammalian muscle and injure the muscle, the injured muscle does not get repaired. In this way, we know that satellite cells are required for muscle repair. These cells can be identified inside a muscle because they have the protein Pax7 (***Chang NC, Stem Cells in Development and Disease. 2014)***. Mature muscle fibres do not have this protein.

Up until 2017, satellite cells were thought not to exist in fruit flies (***Rai M et al, Mechanisms of Regeneration. 2014)***. Some researchers have said that satellite cells do not exist in insect muscles. Though strange things happen in nature, this was particularly curious.

Therefore, in the lab of Dr K Vijay Raghavan at NCBS, we looked for satellite cells in fruit fly muscle. Unless you have very clear experiments and unquestionable data, it is hard to overturn a belief in science. The great thing about science though is that new and compelling evidence can change beliefs.

Dr Rajesh Gunage, from the same lab, had found a group of cells needed to form adult Drosophila flight muscles, called adult muscle progenitors. Based on what we know about muscles from fruit flies and mammals, it followed that the cells that Rajesh had identified, would/should give rise to Drosophila Satellite cells. He showed very clearly that the daughters of adult muscle progenitors become a part of adult flight muscles and also become cells with single nuclei right adjacent mature muscle fibers. The physical similarity to mammalian satellite cells, even as seen in electron micrographs, was striking. He also found which 'word', technically called a signalling pathway, which these cells use to communicate with their surroundings. This is the Notch pathway.

To convince researchers like us, that a new group of cells has been identified, especially one that is completely unexpected, we had to provide a unique feature of these "satellite" cells, much like Pax7 in mice. This makes complete sense. If I am given a bunch of cells, I have no way of studying specific cells in the collection without a 'marker'. Imagine identifying one person on a bustling crowded platform at a railway station. You need something specific (like a blue shirt and white pants), about one individual to observe what they do.

So, I looked for one protein that is found only in fruit fly satellite cells. If this protein is expressed in satellite cells, it had to be necessary for muscle formation. Logic dictates that if it is found in satellite cells in fruit fly flight muscles, it would also be in cells that contribute to mature muscles, say in larvae or pupae. Though, the requirement of a number of proteins is known for muscle formation, guessing which one would be only in satellite cells was non-trivial. I checked and found only one of the many proteins was seen in adult muscle progenitors and in satellite cells in adults. We even found a small time window in which the cells that will become satellite cells in adults keep this protein, but others lose it. This protein is called Zfh1.

If cells with Zfh1 are like mammalian satellite cells, they would increase in number if the muscle is injured and help with repair. We used a simple pin prick to damage flight muscle in adult Drosophila. We then quickly showed that daughters of cells with Zfh1 in adult Drosophila flight muscles divide and might help with repair after injury.

So, at Dr Vijay Raghavan's lab in NCBS were the first to identify cells in adult Drosophila flight muscles that maybe functional equivalents of satellite cells in mammals (***Chaturvedi D et al, eLife 2017)***. This opens up new possibilities to answer fundamental questions about muscle maintenance. We now have a way of studying their behaviour in living tissue inside an animal. That had been difficult so far. We sincerely hope our findings about muscle maintenance and repair in fruit flies will someday help patients with genetic muscle diseases. In scientific terms, this information changes how we think about muscle formation and muscle repair in the tree of evolution.