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Researchers Identify Fruit Fly Intestinal Stem Cells

Howard Hughes Medical Institute researchers have identified stem cells in the gut of the fruit fly—a finding that may lead to new insights into digestive diseases, intestinal cancers, and the infection strategies used by insect-borne parasites. The new discovery puts to rest a scientific debate over whether invertebrates have gut stem cells.

From an evolutionary perspective, the discovery of immature cells that differentiate into multiple types of gut cells suggests that the digestive tract of the fruit fly is likely to be a more similar, albeit simpler, version of that found in humans.

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— Allan C. Spradling

As a result of the new findings, scientists now envision using the fruit fly—a genetically malleable model organism—to explore normal and pathogenic regeneration of the digestive tract in ways that were not available before.

The two HHMI research teams reported their independent findings on December 7, 2005, in two online research articles published in the journal *Nature*. The two groups were led by Craig Micchelli and HHMI investigator Norbert Perrimon, who are both at Harvard Medical School, and Benjamin Ohlstein and HHMI investigator Allan C. Spradling, who are both at the Carnegie Institution of Washington.

Although *Drosophila* stem cells have proven invaluable in studying regenerating tissues such as the reproductive system, they had not been found in the digestive tract. In fact, many scientists believed that the fly intestinal tract was a relatively stable tissue, in contrast to those of humans and mice, which undergo constant turnover to replace cells damaged or lost to abrasion from ingested foods or exposure to toxins and pathogens.

To find out if the digestive tract of the fly harbored stem cells, both research groups began by using cell-tagging tracers. In this way, they determined that

the *Drosophila* gut appeared to contain different types of cells—those that nestled against muscle tissue of the gut wall and those that extended into the interior space. Both groups used genetic techniques to label cell lineages in the *Drosophila* gut. Those approaches revealed that certain cells divided constantly, whereas others had differentiated into mature gut cells and ceased dividing.

Ohlstein and Spradling began their studies by marking major intestinal cell types with specific antibodies. That labeling revealed “cell nests” in the intestine that included one type of cell that made extensive contact with the intestinal cell wall, and another that did not. Further labeling to determine the proliferative behavior of these cells revealed intestinal stem cells (ISCs) that could divide indefinitely. In contrast, the non-stem cells they labeled did not continue to divide.

“We found that the rate of stem cell proliferation and cell turnover depended on how much food the flies were eating,” said Spradling. “If they got old moldy food—that had a bad effect also. So we knew that this proliferation was responsive to factors that affected the gut and the physiological processes, which tells us there is a lot of interesting biology left to do to understand this system.”

Ohlstein's and Spradling's study also revealed a high level of programmed cell death, or apoptosis, in the gut - similar to what is seen in the intestines of vertebrates. “We found that balancing this high level of stem cell activity was a rapid turnover of all the cells in the gut, with the whole gut essentially turning over every week. This is very surprising based on what people previously believed about a stable intestine in the fly, and it allows to understand why there is so much stem cell activity,” said Spradling.

Their analysis showed that *Drosophila* ISCs, like those of vertebrates, are multipotent, meaning they have the potential to give rise to multiple types of intestinal cells.

Another important observation emerged when Ohlstein and Spradling explored the function of the master cell regulatory protein Notch. They chose to investigate Notch because it was known to be involved in control of cell differentiation. By knocking out Notch activity in the ISCs, they found that the protein affected the cells' ability to make the correct mix of daughter cells. Furthermore, they found that Notch was also required for the continued differentiation of the daughter cells, a role that had not been identified previously in vertebrates.

Micchelli and Perrimon began their study by characterizing the cellular organization of the *Drosophila* gastrointestinal tract. This analysis revealed two general cell types in the gut: epithelial cells that lined the gut, which had large, oval nuclei; and a second class of cells lying in close apposition to the muscle surrounding the gut, whose nuclei were smaller. A series of cell proliferation assays revealed that only the cells with the small nuclei were undergoing cell division.

To further characterize the population of cells with small nuclei, the researchers initiated an expression screen to identify specific molecular markers. Identification of specific molecular markers for the small cells enabled the scientists to further subdivide this population on the basis of a distinct molecular signature. Cell lineage-tracing experiments next revealed that the differentiated cells of the gut are related by lineage and arise from a common stem cell progenitor.

Micchelli and Perrimon next explored the role of Notch signaling in the ISCs. Using a technique that allowed both spatial and temporal control of gene expression, they demonstrated that Notch is required specifically in stem cells to limit their proliferation. Interestingly, reduction of Notch in stem cells led to the generation of far more stem cells in the gut and the appearance of tumor-like masses, said Micchelli. Their experiments showed that active proliferation was the default state for the ISCs, which Notch could switch off.

The two research groups said that discovery of the ISCs make *Drosophila* a far more powerful animal model for basic studies of the machinery of stem cell biology. “The *Drosophila* stem cell system is much like the more complicated vertebrate system, but stripped down just to the essentials. So, it's much easier to work out the basic biology of what information one cell conveys to another,” Spradling said.

Micchelli said that “gut stem cells have not been unambiguously identified in mammalian systems, so with the discovery of *Drosophila* ISCs, we have the first characterization of gut in a tractable molecular system.”

Both research groups emphasized that the discovery of *Drosophila* ISCs will have important implications for basic understanding of cancers, digestive disorders, and the action of parasites.

“There are a number of gastrointestinal diseases and cancers whose molecular basis is only poorly understood. The *Drosophila* system will enable us to rapidly identify the genes that normally regulate self renewal. Ultimately, we seek to shed light on gastrointestinal disease by understanding the consequences that mutant forms of these genes have on tissue homeostasis,” said Micchelli.

Spradling emphasized the importance of the new findings in understanding how parasites such as malaria trick their mosquito carrier, or vector, into passing them from the gut to the salivary gland, where the parasite is transmitted by biting a victim. “Knowing that there are stem cells that undergo constant turnover will be extremely valuable in understanding how parasites defeat the defenses of the vector gut and get into the body cavity, and then migrate to the salivary glands,” he said. “These findings give us new ideas and new tools to attack these questions.”

Spradling and Ohlstein are optimistic that studying intestinal stem cells will enable detailed understanding of the genetic aberrations in specific intestinal cells that can lead to tumors. “The relative simplicity of the *Drosophila* gut will make it easier to study these tumors as well as to screen for drugs that

affect them,” added Ohlstein.