

## New study documents use of hormone progesterone in simple microscopic aquatic animals

June 14 2010



This confocal fluorescence microscope image shows a female rotifer treated with a fluorescent progesterone probe. Credit: Image: Paige Stout

A new study shows that humans and tiny aquatic animals known as rotifers have something important in common when it comes to sex.

Barely visible without a microscope, rotifers eat algae and serve primarily as food for baby fish. But the females of certain rotifer species can do something quite unusual: they can reproduce asexually by



creating clones of themselves, or they can initiate a process that allows sexual reproduction by producing male rotifers.

The chemical mediator for this change from asexual to sexual reproduction turns out to be progesterone - a simple molecule that also plays a vital role in regulating reproduction and <u>sexual development</u> in humans and many other species. Finding this sex steroid and its receptor in simple rotifers suggests that the progesterone signaling technique dates back hundreds of millions of years.

"This has really important evolutionary implications," said Julia Kubanek, a professor in the School of Biology at the Georgia Institute of Technology and one of the study's principal authors. "Our study shows that the identical steroid molecule found in humans and rotifers is used for two very different aspects of reproduction."

Sponsored by the National Science Foundation, the research was scheduled to be published June 14, 2010, in the early online edition of the journal <u>Proceedings of the National Academy of Sciences</u>. The study is believed to be the first to document the use of progesterone in the lineage of simple animals that includes rotifers - and has been largely unchanged for millions of years.

Most animals reproduce sexually, a method that makes a species more adaptable by facilitating the elimination of bad genes and creating potentially beneficial new gene combinations. Very simple organisms, such as bacteria, reproduce through cell division and obtain new genetic material from the environment.

The rotifer species Brachionus manjavacas is somewhere in between. During most of the year, the rotifer population consists only of females, which reproduce by creating clones of themselves. But when unfavorable environmental conditions threaten - such as the loss of algae food - about



a third of the rotifer population switches to sexual reproduction, which is the only way the creatures can produce eggs able to survive through a long winter.

Kubanek and her collaborators wanted to understand what was triggering that change, which begins with the production of male rotifers. The switching appears to depend on a pheromone protein that the rotifers release into the water to indicate that other rotifers are nearby. When the rotifer population grows large enough to create a significant concentration of that protein, females start laying eggs that can develop as males. A population large enough to do that usually doesn't build up until fall in North America - when winter and the end of the algae food supply are near.

"The rotifers are pretty good at figuring out when conditions are getting bad and when it's time to produce males, have sex, make these overwintering eggs and call it a day," Kubanek said. "They are really making some fairly sophisticated decisions about when to have sex, and when to go it alone."

To understand the complex process, the researchers combined several different research techniques to piece the puzzle together.

Professor Terry Snell in Georgia Tech's School of Biology, along with his students and collaborators at Woods Hole Oceanographic Institution, studied the partially-sequenced genome of the rotifer and located a receptor for a progesterone-like molecule. Then, using a fluorescent probe developed by James La Clair of the Xenobe Research Institute in San Diego, Paige Stout, a Ph.D. student in Georgia Tech's School of Chemistry and Biochemistry showed that progesterone binds to a receptor in the reproductive systems of female rotifers.

The researchers gained further evidence of progesterone receptor's



presence through the use of affinity chromatography, which used the same probe system to extract the receptor from a mixture of proteins contained in the rotifers. Further, mass spectrometry indicated the presence of progesterone at parts-per-billion levels in rotifer mass.

Finally, Snell and School of Biology research scientist Tonya Shearer demonstrated a direct connection between the hormone and the switch to sexual reproduction in the rotifers. They used RNA interference (RNAi) technology to silence the gene controlling the progesterone receptor, which reduced by nearly two-thirds the number of animals switching over to sexual reproduction in response to the progesterone signal.

"This suggests that progesterone is important in this switch from asexual to <u>sexual reproduction</u>," Stout said. "Progesterone is certainly involved in the process, though there may be more going on in a cascade process that we haven't yet seen."

Beyond explaining how chemical signaling mechanisms crucial to both rotifers and humans evolved over time, the research can also help researchers understand the interplay between the environment, metabolism, hormones and behaviors.

"We can learn things from working with rotifers that touch upon human biology, including - in this case - the universality of steroids in reproduction and how steroids are used differently in different animals," Kubanek said. "When we experiment with rotifers in the lab, we can manipulate their behavior, physiology and metabolic pathways to determine how these steroids are made and used."

Though the researchers were looking for progesterone in the rotifers, finding the same molecule that is essential to humans still came as a surprise.



"It's a very complex system for such a simple organism," said Stout, whose primary research focuses on complex potential pharmaceutical compounds produced by marine organisms such as seaweeds. "Though we were certainly looking for it, I was surprised to see <u>progesterone</u> and not some derivative of it."

## Provided by Georgia Institute of Technology

Citation: New study documents use of hormone progesterone in simple microscopic aquatic animals (2010, June 14) retrieved 1 May 2024 from <u>https://phys.org/news/2010-06-documents-hormone-progesterone-simple-microscopic.html</u>

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