

Profile of John J. Eppig

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On a spring afternoon in 1955, a 12-year-old boy hurried home on the well-trod path from school. As he dashed past the ponds of Long Island's North Shore and rounded Shelter Rock, his thoughts turned to the collection of jars waiting in his bedroom, each one filled with water from those very pools. For weeks, he had cultivated protozoan life in the jars, meticulously documenting their growth in various conditions using a microscope given to him by his grandfather. Full of anticipation, he ran up the front walk and into the house. Gently, he opened his bedroom door—and gasped. The jars were gone.

“My mother was afraid there was going to be some horrible pathogen generated in my room,” says John Eppig, a professor at The Jackson Laboratory (JAX) in Bar Harbor, Maine, and a recently elected member of the National Academy of Sciences (NAS). “She decided on a spur of the moment that this experiment was terminated.” Little did she know that her son’s curiosity about the natural world would later enable researchers to create viable embryos using cell culture.

Over his 48-year career, Eppig has garnered countless honors and published hundreds of articles, all derived from a single inspiration: Nature. “I like to look in the tidal pools and watch the birds—the osprey diving in front of our house and the bald eagles swooping down to grab fish,” Eppig says. “All of those animals have to reproduce. What strategies do they use? Would the strategies used in mammalian ovaries and testes work in other species? What can we learn from Mother Nature?”

In his Inaugural Article (1), Eppig explores these questions as they relate to the development of immature egg cells, or oocytes. His work reveals that a protein called meiosis arrest female-1 singlehandedly performs a variety of functions in oogenesis that require the work of several molecules in the analogous process of spermatogenesis. The findings may help explain how the genomic integrity of the female germ line is protected during the carefully orchestrated events of sexual reproduction.

From the Shores of Long Island to the Woods of Tennessee

John Eppig was born in 1943, the second child of a pediatrician father and a homemaker mother, who had earned her master’s degree in English before devoting herself to family life. His father had hoped Eppig might follow in his career footsteps, but it soon became apparent that his son preferred biology to medicine.

Eppig attended Villanova University in Villanova, PA, where he excelled in history and biochemistry but struggled with math. Eppig barely passed physics, and only with the help of his roommate, a bright engineering student. “But I loved biology,” he recalls. “I became more and more interested in the phenomenon of metamorphosis in insects and frogs.”

A college professor put Eppig in touch with Gardner Lynn, an expert in amphibian metamorphosis at Catholic University of America in Washington, DC. After two years of graduate work in Lynn’s laboratory, Eppig applied to the Oak Ridge Associated Universities predoctoral program, where he hoped to complete his dissertation research at the Department of Energy’s Oak Ridge National Laboratory in Oak Ridge, Tennessee.

Acceptance to the prestigious program seemed imminent, thanks to Lynn’s ties to Norman Anderson, a respected biophysicist at Oak Ridge who had agreed to mentor Eppig. However, there was a catch: Eppig had to pass a mathematics proficiency test. Although he was stumped by most questions on the examination, Eppig later learned he had scored higher than most engineers. “The only thing I’ve been able to figure is that my scores were somehow switched with those of the only other person taking the test,” Eppig says. “He had slide rules dangling from his belt and seven different colored pens in his pocket. He’s probably still wondering how he flunked that test.”

The Molecular Anatomy Program at Oak Ridge afforded Eppig a laboratory of his own and an uncommon level of freedom. His fascination with amphibian metamorphosis gave way to an interest in pigment patterning during early amphibian development. Keen to discover how the pigment was synthesized,



Eppig at the helm of his Pisces 21 sailboat, *Keepah*. Image courtesy of Dan Farrenkopf.

Eppig performed a series of electron microscopy experiments and biophysical analyses on the developing eyes of newts, tadpoles, and frog larvae (2). He often drove to the Cumberland Mountains to collect female amphibians and their eggs for his research. “I loved wading around old abandoned strip mines that had been filled with water and become swamps with mean-looking snakes,” he says.

Over time, his reasoning skills became apparent. From Anderson, Eppig learned a valuable lesson, one that he has since passed on to every trainee in his laboratory. “Imagine yourself as Mother Nature,” he says. “Ask yourself, ‘What is nature’s goal and the simplest way to achieve it?’”

Eppig remained at Oak Ridge for his postdoctoral work, moving to the laboratory of James Dumont at the Biology Division to study the development of pigmentation in the oocytes of African clawed frogs (*Xenopus laevis*) and other amphibians. “I was very interested in the cell biology and endocrinology controlling this process,” Eppig recalls. He began developing culture systems for *Xenopus* oocytes and eventually became more interested in the oocytes than the pigment (3). “The rest is history,” he says of his career path.

Onward and Northward

As he neared the end of his postdoctoral work and began searching for a faculty position, the job market was bleak. “If you look in the back issues of *Science* and *Nature* you’ll

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member’s Inaugural Article on page 18653 in issue 46 of volume 109.

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see that there were no jobs available in 1972. And if there was a job advertised, there would be 500 applicants,” he says. Eppig soon realized he was searching in the wrong section of *Science*. “I decided to look in the obituaries,” he says. “I wrote to places where there was an obituary for a biologist. I would say, ‘I see that you potentially have an opening.’”

By the time Eppig received a positive reply, he had already landed a position on the faculty of Brooklyn College at the City University of New York, where a new science building had opened. Eppig befriended another City University of New York researcher, Edward Leiter, who spent summers at JAX. During a visit to Leiter’s laboratory in Bar Harbor, a famed stem cell researcher by the name of Roy Stevens invited Eppig to return the following year as a visiting investigator. Eppig accepted, and came back to study teratoma development in mice with Stevens during the summer of 1975 (4). At the end of the stint, Eppig was invited to stay. The stay lasted until his recent retirement in July 2013.

“Very little had been done to study oocyte development in mammals at that time,” Eppig says. “I came to JAX convinced that as far as oocytes were concerned, mice were just warm, furry frogs. And that turned out to be quite wrong.”

The first question Eppig tackled upon arriving at JAX was one researchers had struggled with for years: whether mouse oocytes can undergo true meiosis in cell culture and become functional gametes capable of fertilization and embryonic development. In the 1930s, researchers had presented evidence that fully grown oocytes, when removed from their follicles and placed into culture, would spontaneously undergo the first meiotic division without the hormones that normally drive the process. In the years since, however, no one had succeeded in fertilizing culture-grown oocytes; many dismissed the phenomenon of spontaneous meiosis as an artifact. “They thought it was just the oocytes going nuts, that they weren’t really undergoing meiosis or becoming functional gametes,” Eppig says. “But if I was going to have a career where we used spontaneous maturation as a vehicle for understanding the regulation of meiosis, we had to show that you could produce normal animals from those in vitro-matured oocytes.”

Waters of Life

Eppig’s laboratory was down the hall from a pair of star researchers in in vitro fertilization, as a result of their work on the early development of mammalian embryos in culture.

“Wes Whitten was an Australian expatriate who, on the side, studied fox pheromones by going out to collect yellow snow,” Eppig says. “And Peter Hoppe was a local good ol’ boy who made good in the IVF [in vitro fertilization] world. They had this common paranoia about their culture systems, and that was one of the secrets of getting it all to work.”

Whitten and Hoppe taught Eppig’s group an important trick. “We went down to look at what those guys did, and we were just stunned,” Eppig says. “They were going out and collecting water from nearby springs or lakes and distilling it themselves. They weren’t using any store-bought culture media, but amazingly they used distilled fresh Maine spring water to make their media from scratch. So that’s what we did, and that’s what we still do.”

Members of Eppig’s laboratory learned to be “incredibly paranoid” about every aspect of the water distillation process, from the cleanliness of glassware to the purity of the components and everything in between. In the weeks that followed, Eppig’s laboratory laid to rest the debate about in vitro oocyte maturation, showing that fully grown mouse oocytes could in fact be removed from their follicles and allowed to mature—as well as undergo spontaneous meiosis—in culture. From that time, every drop of water used to make culture medium in Eppig’s laboratory was collected from Sieur de Monts Spring in Acadia National Park. “When any of my friends around the world were having problems with their culture systems, we would either send them water or culture medium, knowing that it will work.”

The water distillation technique provided the impetus for more than 30 years of research. In addition to their molecular studies, the laboratory began developing culture systems for potential use in human medicine and agricultural livestock.

In 1977, Eppig published a report in *Developmental Biology* that described tools for studying mouse oocyte development in various cell culture systems (5). The article laid the groundwork for modern understanding of mammalian oocyte development, showing that oocyte growth and development in culture requires an intimate connection to the surrounding cells, known as granulosa cells, and that the relationships between the oocyte, granulosa cells, and somatic—or nonreproductive—cells are essential for oocyte maturation. At the time, researchers knew that the oocyte could not metabolize certain energy sources, and that the surrounding granulosa cells provided the missing metabolites. “But the concept at the time

was that the oocyte was a passive cell that received all of these goodies owing to the generosity of the granulosa cells,” Eppig recalls.

It wasn’t until the mid-2000s that Eppig’s laboratory realized that the oocyte commands granulosa cells to control their development in a way that benefits the oocyte itself (6). “To make up for its own metabolic deficiencies, the oocyte outsources those metabolic processes to the surrounding granulosa cells, and secretes factors that encourage somatic cells to promote those metabolic processes in the granulosa cells. That provides the oocyte the things that it was missing,” Eppig says.

Oogenesis from the Beginning

Although Eppig’s experiments initially revolved around fully grown oocytes, the laboratory was progressively retracing the steps of oocyte and follicular development, working backward to the origins of oogenesis. By the late 1980s, Eppig’s group was studying half-grown oocytes; by the mid-1990s they were on the brink of studying oocytes in primordial follicles: in other words, cells present at birth. Achieving full oocyte development in culture, however, was no trivial task. “You can’t just isolate primordial follicles and get them to develop in culture. It just doesn’t work,” Eppig says. The group decided to harvest and culture ovaries from newborn mice, then extract the oocyte-granulosa cell complexes of the half-grown oocytes. Eventually, the group induced 20-micrometer-wide oocytes to grow into 70-micrometer-wide oocytes.

A small number of the oocytes were successfully fertilized, and Marilyn O’Brien, research assistant in Eppig’s laboratory, transferred them to pseudopregnant foster mothers. “Lo and behold, Marilyn came in one morning, and here was this tiny, really undersized baby. But it was alive,” Eppig says. “We were totally shocked because we had transferred almost 300 embryos and we did not have high hopes.”

The baby was named Eggbert, a name that research assistant Karen Wigglesworth, crafted from “egg” and “birth” (7). “We were stunned that it lived, and that mommy took care of it,” he recalls. However, Eggbert was not a normal mouse. As an adult, he was obese, had faulty organs, and had various neurological problems caused by a large brain cavity. The Eppig laboratory spent the next few years optimizing the conditions of oocyte development in the hopes of producing a healthy mouse. The researchers tried adding epidermal growth factor or follicle-stimulating hormone, or various other factors that might, at various time points, assist the process. The

group modified the culture media. “We tried a number of things, really pretty much without any rational basis except the fact that maybe they would work,” he says. “In the end, we made a lot of Eggberts and they all turned out to be normal as far as we could tell. It just goes to show the importance of the culture conditions” (8).

Eppig’s laboratory has since experimented with aggregated chimeric ovaries, which allow researchers to tease apart the roles of somatic and germ cells in various disease phenotypes. “One of the things that really interested us was the question of who is in charge of follicular development. Is it the oocyte or the somatic cells?” In a PNAS report, the group used aggregated chimeric ovaries to answer this question, showing that the oocyte determines the rate of follicular development (9).

In recent years, Eppig’s attention has turned toward a search for the factors in the follicle that normally prevent oocytes from undergoing spontaneous meiosis. Together with Eppig’s first postdoctoral researcher, biologist Stephen Downs, and fellow NAS member and biochemist Doug Coleman, Eppig reported in 1985 that one such factor was a purine called hypoxanthine (10). However, in 2010 his laboratory published a *Science* paper describing another such substance in the follicle, called natriuretic

peptide C (NPPC), encoded by the *Nppc* gene (11).

Recently, the group has been trying to determine how factors like hypoxanthine and NPPC might interact in the regulation of meiosis. The results, published in PNAS (12), reveal that signals from the oocyte itself promote the production of meiosis-arresting cGMP by its surrounding somatic cells. The signals from the oocyte stimulate the metabolic pathway in the somatic cells, particularly two key enzymes—natriuretic peptide receptor 2 and inosine monophosphate dehydrogenase—needed to produce cGMP. This mechanism underlies the follicle’s ability to prevent meiosis

until both the follicle and the oocyte are fully developed. As Eppig says, “the oocyte is regulating its own meiosis.”

During his first few years in graduate school, Eppig gave serious thought to a career in insect physiology before gravitating toward metamorphosis and oogenesis. “Now at the NAS I’m a member of Section 61 [Animal, Nutritional, and Applied Microbial Sciences], which includes a lot of entomologists and insect physiologists. And I almost feel like things have come full circle. I’m sitting here among insect physiologists who have reached the pinnacle of their careers and I wonder: If I had followed that path, would I be here now?”

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