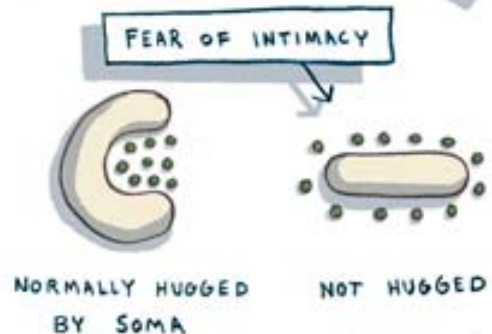
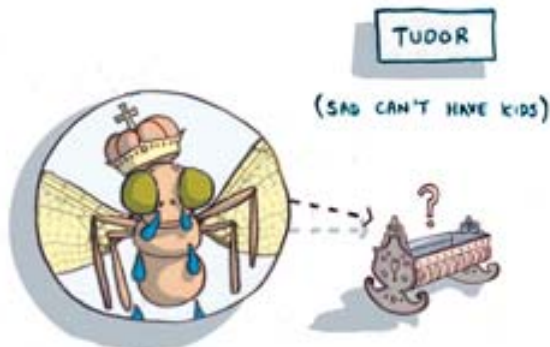
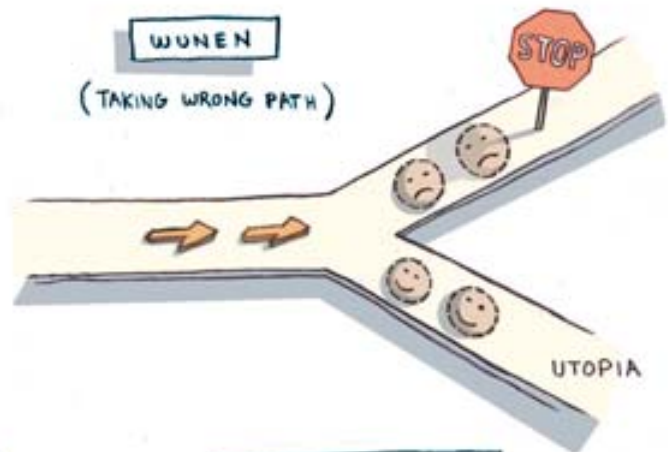
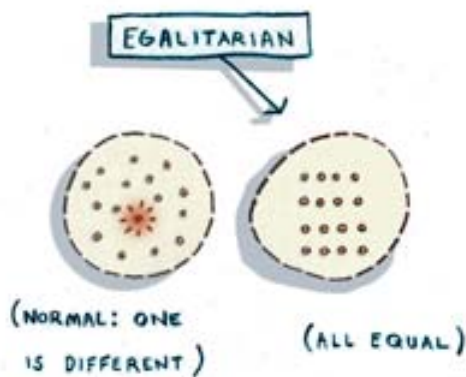
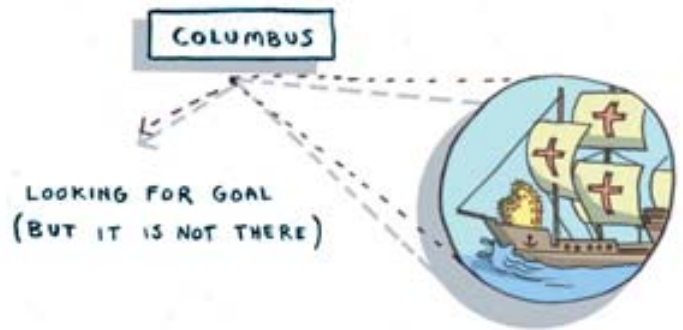


# Germ Cells Are Forever

THE QUEST TO UNDERSTAND THE ONLY CELL



BY VIVIEN MARX

ILLUSTRATIONS BY LEIF PARSONS



## THAT CAN LINK GENERATIONS

- ◀ Genes dictate the development of germ cells, which give rise to sperm or egg and are therefore crucial for reproduction and development. Fruit fly scientists pick quirky names such as *oskar*, *tudor*, *columbus*, *egalitarian*, and *fear of intimacy*, to help them remember the function of each gene.

**T**OBİ'S MOIST, BROWN EYES turn quickly from pensive, to curious, to happy, to sleepy. Then again, these are superficial observations, and may be completely wrong. Understanding Tobi would warrant careful, long-term observation and tests. And an important descriptor was left out: Tobi is a dog, mostly beagle. He is Dr. Ruth Lehmann's dog, whose trusted companionship she enjoys after a long day or, often, a late night in the lab.

Developmental biologists like Ruth Lehmann, Ph.D., the Julius Raynes Professor of Developmental Genetics, Director of the new Helen L. & Martin S. Kimmel Center for Stem Cell Biology, and Howard Hughes Medical Institute Investigator, use a classic tool—their eyes—to carefully observe and chronicle change. She and her 19 lab members also apply genetics, cell biology, biophysics, biochemistry, and microscopy to analyze and parcel out what is behind the changes they observe.

During one recent weekly meeting, the researchers collectively compared subtle changes in images of snow pea-shaped embryos. More experiments were devised to analyze what may be driving the changes observed. The researchers presented their work to one another to collect suggestions and critical remarks. Scientific concepts, along with quirky gene names, such as *oskar*, *nanos*, *wunen*, and *fear of intimacy*, bounced around the room.

Fruit fly scientists pick colorful names to help them remember a gene's function. *Fear of intimacy*, for example, is involved in forming the gonad, where eggs or sperm reside.

Dr. Lehmann has become internationally known for her ambitious quest to understand an entire crucial phase of embryonic development: the emergence, formation, and migration of germ cells. Germ cells are universalists of sorts because they can become either egg or sperm and, in turn, foster an entire organism. Though common to all organisms, they are unique in that they link generations by encapsulating the previous generation in the one that follows. Germ cells are forever, as Dr. Lehmann is wont to say. Studying these cells can yield a better understanding of embryonic development, help to understand reproductive function and disorders, and propel stem cell research.

Germ cells are challenging to study because they don't just emerge and grow in one place. They travel a long, elabo-

varies or testes. A gonad—the ovary or the testis—is made up of germ cells, which give rise to sperm or egg, and somatic cells, which support the germ cells. Getting the germ and somatic cells to the gonad is a just-in-time feat, since they themselves form in different parts of the embryo at different times. This complex behavior has fascinated Dr. Lehmann since graduate school. In the words of the eminent developmental biologist Scott F. Gilbert, Ph.D., “The questions asked by developmental biologists are often questions about becoming rather than being.”

“The work in our lab is having a big influence on understanding germ cell migration,” says Dr. Lehmann. Migration involves movement as well as complex signaling patterns of chronological and spatial cues that choreograph germ cell development. Embryonic development—going from fertilized egg to complex organism in a short amount of time—amazes scientists, who use the words “beautiful,” “awesome,” “ele-

phant's four chromosomes. *Drosophila* generates plenty of progeny, and it's easy and inexpensive to keep in labs. All of which helped Nobel Laureate Thomas Hunt Morgan, the father of modern genetics, to become the first to reveal functional concepts of chromosomes in the early 20th century. Many scientists have advanced his work, including Nobel Laureate Christiane Nüsslein-Volhard, Ph.D., who directs the Max-Planck-Institute for Developmental Biology in Tübingen, Germany, and was Dr. Lehmann's doctoral adviser. It was genetics that got Dr. Lehmann hooked on the fruit fly. “Genetics is the means to analyze function,” she says, “and we want to understand how things work in the living organism.”

After her postdoctoral training at Medical Research Council in Cambridge, England, Dr. Lehmann, a native of Germany, joined the Whitehead Institute of Biomedical Research in Cambridge, Mass., and was appointed Assistant Professor at the

## THE FRUIT FLY IS A SUPERSTAR IN GENETICS AND

rate journey through various parts of the embryo to get to their proper position. Once there, they meet up with the other cells of the body, somatic cells, and together form a set of gonads:

**Dr. Ruth Lehmann**



gant,” and “powerful” to describe this process. The animal Dr. Lehmann studies is not her loyal canine companion, Tobi, but the fruit fly *Drosophila melanogaster*, better known as that insect that hovers annoyingly around spoiling fruit. While the fruit fly is not a mammal, its germ cell development bears important similarities. “The fruit fly lets us study and understand processes that are much more difficult to study in other organisms,” says Dr. Lehmann. Researchers can target their studies of other animals much better because they can build on insights drawn from fruit fly research, she says.

The fruit fly—about 3 millimeters long when fully grown (about half the diameter of a pencil eraser)—is a superstar of genetics and embryology. Scientists can observe its characteristics, see how it functions, and find the genetic underpinnings of both using various techniques to highlight genes on the

Massachusetts Institute of Technology. In 1996 she moved to NYU's Skirball Institute of Biomolecular Medicine, where she is a Howard Hughes Medical Institute Investigator; the institute provides funding for Dr. Lehmann and her lab. Last year, she was elected a foreign associate member of the National Academy of Sciences, a high honor.

Dr. Lehmann says that looking at the embryo yields many surprises. After an egg is fertilized, it divides multiple times. Then comes a phase of forming, reshaping, and in-folding, during which the embryo's parts move into position and develop. Given the enormous complexity that must arise out of simplicity, these rearrangements are carefully controlled. As Dr. Lehmann notes, “There is so much feedback in an organism.”

Researchers have discovered that fruit fly embryos create minute but marked gradients of proteins that act as spatial cues to guide development. Many of



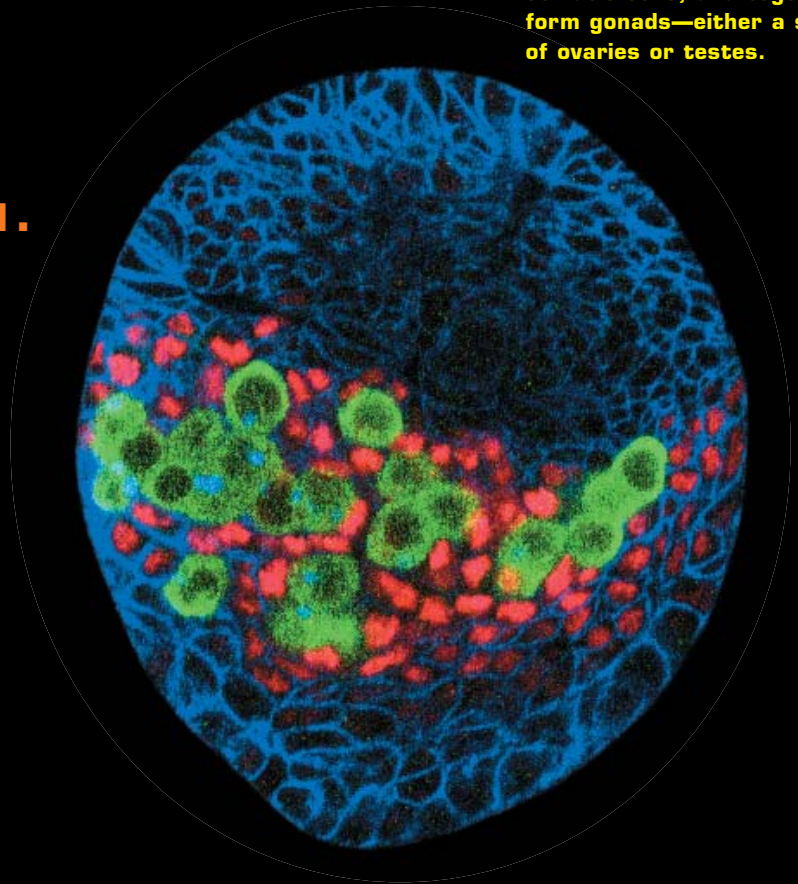
The fruit fly, illustrated at left, and its embryos, shown far right, as seen under low magnification. The germ cells (black dots) have been made visible by staining. It takes about 12 days for a fertilized egg to develop into an adult fly.



A section of the fruit fly ovary (above). Germ cells migrate through the body of the embryo to reach the ovary. Once there, a subset of them becomes stem cells, shown in the structure at the far left of the section. Each stem cell produces a cyst of 16 cells, only one of which develops into an egg, shown in red at far right of the section. The other 15 will become nurse cells, shown in green at far right, which feed the egg.

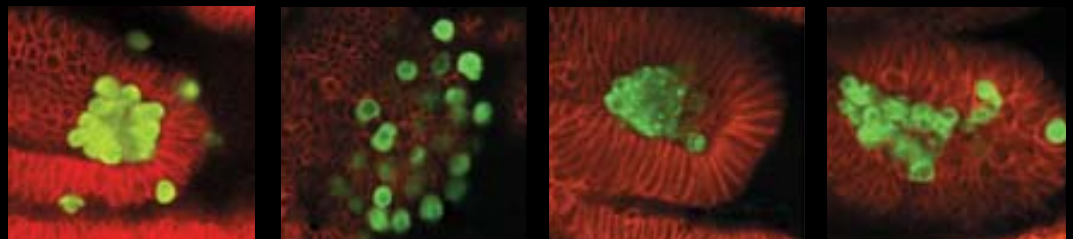
The fruit fly ovary (below). Germ cells, shown in green, can become either sperm or eggs. These cells travel a long way to reach their destination. There, they fuse with other cells, called somatic cells, and together form gonads—either a set of ovaries or testes.

## EMBRYOLOGY RESEARCH.



As part of their normal development, germ cells, shown in green in the sequence below, initially adhere to one another (first image). In the next stage of development, they must separate and disperse (second image).

With live cell imaging and other techniques, Dr. Lehmann's lab identified the gene and protein that help the germ cells separate and disperse. When the cells lack this gene, they stay put (third and fourth images). The mutation is called *tre-1*, for trapped in endoderm-1.



these signals are in the unfertilized egg delivered exclusively through maternal genes. These genes dictate signals that shape the way the fertilized egg, a product of both mother and father, develops. These signaling substances diffuse through the embryo and ensure that the wings, the head, and the internal organs all emerge in the embryo in their predestined places. Earlier in her career, for example, Dr. Lehmann helped to identify previously unknown genetic signals for spatial cues governing the head and tail of the adult-to-be.

Much cell biology has been test tube biology, according to Dr. Lehmann. “We build on those studies,” she says, “but in the end we always want to go back to the embryo to try to understand how the whole embryo works.” It’s what she calls an organismal approach to research. A physical change in the fly relates directly to a genetic one. That “tip of the iceberg,” as Dr. Lehmann calls it, enables researchers to look deeper to see how genes rule function.

Dozens of racks, each containing vials of fruit flies, are stacked in the comfortably warm walk-in room where the insects live. Each vial contains a group being used in an experiment; they are in different stages of development and have differing traits. Globes of yeast, the fly’s favorite food, sit at the bottom of the vials. Researchers take their vials from the racks to the fly room to look at them and set up crosses, in which males and females

Siekhaus, Ph.D., a postdoctoral fellow whose previous research at Stanford and the University of California at Berkeley, was in fruit flies and in yeast. To work with flies, she says, you have to be very good at picking up subtleties and integrating that visual knowledge.

Her specific research involves *Drosophila’s* immune cells, known as hemocytes. They, too, migrate like germ cells, although they disperse differently. Like all of her lab colleagues, Dr. Siekhaus seeks to understand cellular decisions in the context of the environment of the organism. As Dr. Lehmann explains, this scientific approach about organismal context has many implications. For example, many of the genes involved in cancer have been identified. Mutations can be found in all of the body’s tissues, but cancers seem to form in certain tissues and not in others. “The ability of tumor cells to navigate through the blood and lymphatic system and exit it at a certain place is a critical step in metastasis,” she says.

It takes about 12 days for a fertilized egg to develop into an embryo, then a larva, and an adult fly. Dr. Lehmann studies the niche at the rear of the embryo where the germ cells are formed. These cells swim in the germ plasm, the soupy fluid containing proteins that lend the germ cells their special identity. She has identified genes that play a role in this niche.

“In every organism,” she says, “the germ cells are established early, set

When primordial germ cells finish migrating through the body of the embryo, the cells coalesce with the gonad. This is where a number of important decisions are made, explains postdoctoral fellow Lilach Gilboa, Ph.D. Like a busy harbor filled with docking boats, germ cells move into niches provided for them. There, they are transformed into stem cells that can repeatedly produce either egg or sperm. Dr. Gilboa’s latest work is revealing how the ovary “measures itself” to see whether there are enough germ cells present for the adult fly to be fertile, or whether the danger of subfertility looms and more germ cells need to be made.

Understanding such mechanisms can potentially help explain the regenerative abilities of some organs. For example, if part of the human liver is removed, it can grow back. “How does the liver know to grow back, know what is the normal size, and what the right ratio of its different cells are?” asks Dr. Gilboa. Because the tools to study fruit flies are so powerful, she says, these questions can now be addressed, and the answers applied to complex organisms.

One remarkable trait of germ cells is their migration. “They know they are different while they are moving through the body,” says Dr. Lehmann. “On their trek, they could decide to become a muscle cell, but they don’t.”

Primordial germ cells travel in complete silence; their genes are inactivated

## “I LOVE LOOKING AT THOSE CELLS,” SAYS DR. RUTH

are placed in vials together so that they can produce progeny. The fly room is also a gathering place for the scientists, which contrasts with their solitary bench work.

The scientists look at the flies laid out on a pad under the dissecting microscope. The flies are asleep; carbon dioxide is piped in and diffused through the pad, but they twitch occasionally, and can be pushed around gently with a thin brush. “I really love studying questions about genetics and how cells interact. The fly is great for that,” says Daria

aside, and treated differently from other cells.” Dr. Lehmann seeks to understand the precise nature of what makes these cells so different. Some primordial germ cells develop directly into either egg or sperm when in the gonad. Others take on a special identity, becoming stem cells. They are rejuvenators, producing sperm or eggs continually as their livelihood. “How do they know, ‘You are ok, you can keep making an egg, or you can make sperm?’ We don’t know that yet.”

and do not produce protein. What they are following is an intrinsic program set up by maternal genes, placed in the unfertilized egg, and protected in the germ plasm. As postdoctoral fellow Andrew Renault, Ph.D., phrases it, the silence is as if “they are meant to just concentrate on their journey.”

Dr. Renault, who came to Dr. Lehmann’s lab from Oxford University, is deciphering another phase in germ cell development: namely, the cues that guide the cells along their migrating path. He

studies mutations in genes called *wunen* and *wunen2*, which create a biologically damaging situation for the embryo. “The germ cells don’t have any idea where to go,” he says, “and they scatter over the whole posterior of the embryo.” Flies with this mutation would be infertile.

*Wunen* in flies, which has a mammalian counterpart, is found and expressed in somatic tissue cells. As Dr. Renault explains, *wunen* appears to pave the way for migrating germ cells to stay on track and avoid somatic tissue until their final destination. Both *wunen* genes deliver the blueprint for enzymes that catalyze a chemical reaction with substances in the cell called lipid phosphates. Where this reaction occurs, lipid phosphate is depleted. “We think this leads to a gradient, a diffusible signal in the embryo,” says Dr. Renault. The germ cells follow this trail and avoid the locations where that specific chemical reaction has taken place. Researchers have not yet identified the lipid phosphate but this is one current focus of their research.

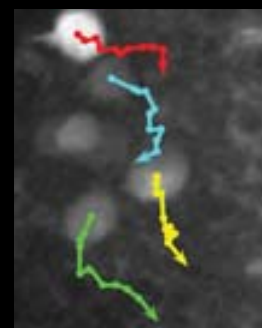
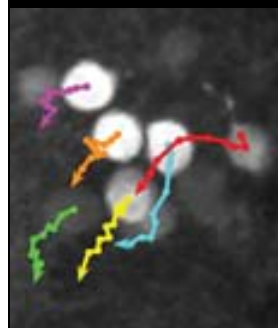
These types of questions have important ramifications. Normally, germ cells in fruit flies set off on their approximately three-hour journey to the gonad, and at a key moment split into two groups along the midline of the embryo, moving toward either the left or right ovary or testis. In humans, germ cells that get derailed are known to give rise to teratomas, germline can-

look at a cell in the live tissue and really see what is wrong with it,” says lab member Prabhat S. Kunwar. “That’s really nice.” The Nepalese scientist, who is writing his doctoral dissertation, chose NYU for its strong developmental biology program. During his four years in the lab, he has already made a mark by identifying an important facet of germ cell migration. Initially, he says, primordial germ cells adhere like grapes in a bunch. They are surrounded by another cell type, epithelial cells. As germ cells set out to migrate, they must disperse and pass single-file through junctions between the epithelial cells.

With live-cell imaging and other techniques, Kunwar identified the gene and protein that help the germ cells separate and disperse. Without this gene, cells stay put, which is why it is called *tre-1*, for trapped in endoderm-1. Intriguingly, the molecule is related to one that is important for the movement of white blood cells in humans. But *tre-1* is hardly the entire story. As Dr. Lehmann explains, “We are working to find the factors that set the germ cells in motion. How do they know when to stop? We are just beginning to look at that.”

There are many mysteries yet to be solved, and they will require many years of rigorous work. Those who work with her admire Dr. Lehmann for her discipline, compassionate mentoring, and enormous experience. She is not just interested in the complicated signals

During another step in their migration, germ cells, shown in pink, leave the gut (top image) and continue on their path toward the gonad (bottom image).



Using special imaging techniques, scientists in Dr. Lehmann’s lab track germ cell movement (above). Chemical cues guide the cells along their migrating path. When there are mutations in the two genes called *wunen*, those chemical cues disappear and the germ cells no longer have a path to follow.

PHOTOGRAPHY: DR. HIROKO SANO (TOP AND CENTER); LESLIE PUSATERI (BOTTOM)

## LEHMANN, DEVELOPMENTAL BIOLOGIST.

“ I LIKE TO SORT FLIES.”

cers that develop in young adults.

As Dr. Lehmann explains, how *wunen* is regulated seems to affect every step of germ cell migration. “It is incredibly complicated how they move,” she says, “through tissues, over tissues, under tissues.” *Wunen*’s fine-tuning allows germ cells to travel from one location to another in the embryo.

Imaging technology, such as deep tissue live-cell imaging with time-lapse photography on a microscope, is an integral part of the lab’s work. “You can

that determine how germ cells develop. She is also keen on mentoring scientists in her lab and helping them to learn how to become independent and creative researchers, enabling them to run their own labs in the future.

As the principal investigator, Dr. Lehmann doesn’t just strategize about the direction of the research. She, too, puts in her hours in the fly room. “I love looking at those cells,” she says. “The germ cells, the embryos, the staining. I like to sort flies.”

As they are formed, germ cells bud off to the side of the embryo (right). Germ cells are special in all organisms—whether flies, mice, or humans. These cells develop early, are set aside, and function differently from other cells.

