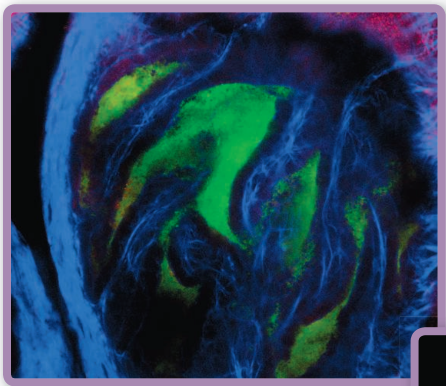
How Do Microbes Shape Animal Development?

As the other mysteries in this package attest, developmental biologists have long had their hands full trying to fathom the transformation of a single cell into a full-fledged adult animal. They have concerned themselves primarily with deciphering the internal, genetically guided programs that take each species through specific stages to come out the right size and shape with a predetermined number of limbs, fins, eyes, and noses. But they were missing an elephant in the room, says Margaret McFall-Ngai, a developmental biologist at the University of Wisconsin, Madison: The world of microbes that live in, on, and around every animal.



The more she and other scientists get to know this world, the more they realize how big an influence microbes have on all aspects of animal and plant life—and not just as infectious pathogens. Take McFall-Ngai's research focus, the Hawaiian bobtail squid *Euprymna scolopes*. A nighttime hunter, it has evolved a way to acquire the bioluminescing bacterium *Vibrio fischeri* from surrounding seawater to light its underside so that predators below don't see its shadow in the moonlight. Squid embryos temporarily develop a mucus-laden ciliated patch inside the body cavity, where *Vibrio* selectively accumulate and eventually migrate into crypts destined to become the squid's so-called light organ. The presence of the bacteria affects squid gene activity, causing the ciliated patch to disintegrate and the light organ to differentiate. If there are no bacteria, the light organ fails to fully develop.

This example of *Vibrio*s as midwives for the formation of an animal organ raises the provocative and, until recently, largely unaddressed question: How much do microbes shape normal development?

Animals and plants have always shared space with bacteria, fungi, viruses, and other microbes, coevolving through many millennia. In the mid-1800s, however, scientists came to view microbes primarily as enemies and fought hard with antibiotics, vaccines, and good hygiene to get the best of them. But the microscopic world is so intertwined with macroscopic life that the idea that each multicellular animal exists as a separate individual defined by its genome is falling by the wayside. There is a growing realization that microbes and their genes are partners in each animal's journey from egg through adulthood. "What we understand to be the 'individual' develops as a consortium of animal cells and microbes," says Scott Gilbert, a developmental biologist from Swarthmore College in Pennsylvania.

Partners in development. The developing light organ (*below*) of the bobtail squid (*right*) temporarily has mucus-covered surfaces to gather symbiotic bacteria (*left*, green).

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"Microbes came before us, so all development that takes place in all organisms has basically been taking place in the presence of the microbiota," adds Sven Pettersson of the Karolinska Institute in Stockholm.

The evidence for coevolution in developmental processes is coming from far corners of the animal kingdom. Whereas marine biologists once thought that drifting larvae of coral, snails, and other oceangoing invertebrates randomly settled down to become adults, they now know that many respond to cues from bacterial biofilms to pick their new homes. And while many animals develop in wombs or eggs apparently free of microbes, they may still rely on microbes to set in motion or complete certain aspects of postnatal development. Like McFall-Ngai's squid, mammals acquire microbial partners after birth and seem to have evolved strategies to encourage the right species to settle in specific places. Human milk, for example, contains complex sugars that infants cannot digest but which promote the growth of intestinal bifidobacteria.

But what do these microbial partners do? Germfree mice have finally allowed researchers to begin addressing this question. These are mice that lack the usual complement of gut bacteria because they are bred and raised in sterile environments and eat sterilized food. Studies of such mice make an increasingly strong case that bifidobacteria and other gut bacteria guide the postnatal maturation of the intestinal and immune systems, and even parts of the brain, in mammals. The microbes turn on mammalian genes important for cellular differentiation and produce metabolic products that may also affect development. Gut-associated lymphoid tissue and the capillary beds of the villi of the intestine fail to adequately develop in germfree mice, for example. With respect to the immune system, mouse studies also show that a polysaccharide produced by the symbiont *Bacteroides fragilis* helps establish the right balance between helper 1 and helper 2 T cells. B cells also need symbiotic bacteria to develop normally.

The evidence for a role for symbionts in the postnatal developing brain is more preliminary but nonetheless intriguing. More and more connections are being found between the gut microbiota and behavior (*Science*, 12 October 2012, p. 198). In 2011, Pettersson and his colleagues tested anxiety levels and locomotor activity in germfree mice and found that the rodents are hyperactive and have a decreased level of anxiety compared with mice with a healthy microbiota. There

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were also differences in the activity of genes associated with motor activity and anxiety. There seems to be a window of opportunity for the microbiota to influence behavior patterns: Colonizing germfree mice with normal mouse microbes negated these differences in young, but not older, mice, they reported.

Some work suggests that gut microbes influence behavior through the vagus nerve, which connects the brain with the digestive system, but Pettersson and others suspect a role for blood-borne bacterial products as well. These products, which make up 10% or more of the metabolites in blood, may extend the reach of the gut microbiota throughout the body.

That realization may mean that prenatal development in mammals isn't as free from microbial influence as everyone has thought. In mammals, the developing fetus is virtually bacteria-free; hence, researchers have focused on finding a role for bacteria in development after birth. Yet blood-borne metabolites from a mother's gut germs could exert an effect on a growing fetus. "That was one of the assumptions, that pregnancy did not involve microbes," Gilbert says. "But it probably does."

As such assumptions are overturned, researchers are addressing new issues. What is the molecular dialogue that enables the microbial world to influence development? How did that dialogue evolve and how often is it a friendly one? "The big questions are now exposed," says Michael Hadfield, a developmental biologist at the University of Hawaii, Manoa. "After all the years we tended to ignore the bacteria, most people who are studying development should be looking for where the bacteria are and what roles they are playing."

–ELIZABETH PENNISI

How Does Fetal Environment Influence Later Health?

Parents pore over their newborn's face, drinking in the fuzz of her eyebrows, the shape of the chin, searching for themselves in her smile. But they're not thinking about what they can't see, and what ultimately matters more: the heft of her heart, the hormones churning from the liver, all those invisible features that influence her health into adulthood.

While their baby's biology of course reflects a mingling of the mother's and father's DNA, there's more to her than that. In a peculiar way, all newborns are "an expression of the mother," in the words of David Barker, a physician and epidemiologist at the University of Southampton in the United Kingdom. He believes that people are shaped, inside and out, by the maternal environment that sustained them before they were born.

In the late 1980s, Barker scrutinized thousands of birth and death certificates of people from Hertfordshire, U.K., and concluded that those whose birth weight fell on the low end of normal were much more likely to die of heart disease as adults. Since then, Barker has promulgated his theory that maternal environment controls a baby's destiny in more ways than we yet understand.

These days, there's broad agreement that the fetal world, the most rapid period of human growth and development, shapes one's risk of future disease, although how much influence it has remains uncertain. A key missing link is in the mechanism. What switches in the fetus, or the placenta that nourishes it, are flipped by a mother's diet or stress levels? In other words, how does fetal environment mold development?

Those exploring this fundamental mystery have at least one intriguing discovery to follow up. No matter what the stressor on the fetus, studies of people and animals suggest that the output is similar: a higher risk of type 2 diabetes, obesity, heart disease, insulin resistance, and high blood pressure. In rodents, "anything that could be a nutritional stressor seems to have the same effect," says Simon Langley-Evans of the University of Nottingham in the United Kingdom, suggesting that the fetus is implementing a universal response to stress, perhaps to ensure its survival.

Prebirth world. The fetal environment correlates with health later on, but researchers are still disentangling exactly how one connects to the other.

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The early focus of the field that Barker spawned was on birth weight, a crude reflection of a fetus's surroundings: Smaller babies tended to reflect poorly nourished or highly stressed mothers. But what a mother eats when she's pregnant is only a small part of the fetal environment, Barker notes. "The mother's body is the product of her lifetime nutrition," he says—and even her own mother's nutrition, too, because most or all of her eggs are formed before birth.

Scientists are now striving for greater sophistication in exploring the black box of the womb. Animal studies have found that without good nutrient flow across the placenta, the offspring responds "by building its organs on the cheap," says Kent Thornburg, a cardiac physiologist at Oregon Health & Science University in Portland. Hearts have fewer muscle cells. Kidneys have fewer nephrons for filtering urine. There's less skeletal muscle in limbs and fewer insulin-producing cells in the pancreas.

Peeling back the layers, scientists are also finding differences in DNA patterns in the offspring, depending on whether their mothers were properly fed or malnourished. One long-running effort examines men and women who developed in utero during the Dutch Hunger Winter of 1944 to 1945, when the Germans cut off food and fuel shipments to part of the Netherlands. A birthday soon