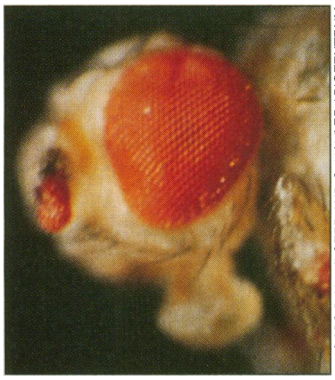
Focusing on the *eyeless* Gene

Activating *eyeless* induces formation of full-fledged eyes in fruit fly wings, legs, and other tissues. This suggests it may be a "master control gene" for eye development

You've heard the expression "He's got eyes in the back of his head." Of course, it isn't meant to be taken literally—people can't sprout eyes on the back of the head, or any other part of their anatomy. But the same can no longer be said for fruit flies. On page 1788, Walter Gehring's group at the University of Basel in Switzerland reports that, when a gene called *eyeless* is turned on in parts of flies where it wouldn't normally be active, the flies grow extra eyes. The Gehring team's experiment produced flies with eyes on their wings, eyes on their legs, and eyes on their antennae. Some flies had as many as 14 fully developed eyes.



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All eyes. The consequences of abnormal *eyeless* activation can be seen in these eyes on the antenna and leg *(right)* of a fruit fly.

"It is really remarkable that you can take a tissue that would normally make a wing or an antenna and by turning on one [gene], make that into a complex thing like the eye," says fruit fly geneticist Gerald Rubin of the University of California (UC), Berkeley. "This is like someone finding a [gene] that would turn a kidney into a liver."

That kind of capability makes *eyeless* the best candidate yet to be the elusive quarry that developmental biologists call a "master control gene," a gene that singlehandedly triggers the formation of an organ or structure. The protein produced by the *eyeless* gene has all the hallmarks of a transcription factor, a protein that turns genes on or off. It apparently "binds to a distinct set of genes that starts the whole process to make eyes," says Larry Zipursky, who studies fruit fly eye development at UC Los Angeles. With the help of *eyeless*, Zipursky says, researchers should be able to "piece together the steps" by which eyes are made.

The work has evolutionary implications as well. Gehring's group has shown that the mouse counterpart of *eyeless* also causes eye formation when it is put into fruit flies. That suggests that the mouse gene functions in the same way as the fly gene, which in turn implies that the gene's role as a master regulator of eye development is very ancient—dating back half a billion years to the common ancestor of flies and mammals.

Gehring's group didn't set out to plumb the history of eye evolution, or even to find the master gene for eye formation. In fact, the team's venture into eye development began "quite accidentally," says Gehring. In 1993, graduate student Rebecca Quiring was searching for fly genes that code for transcription factors. In the course of her search, she pulled out the gene for one such factor, and postdoc Uwe Walldorf found that it mapped to the same chromosomal site as the *eyeless* gene. Although not yet cloned, *eyeless* had been identified years earlier through its effects in genetic studies: When mutated, the gene produces flies with deformed eyes or no eyes at all.

Upon analysis of the gene, Quiring and Walldorf made a startling discovery: The fruit fly *eyeless* gene is very similar to genes that play roles in eye formation in mice and humans. The mouse gene is called *Small eye* because mutations in one of its two copies yield underdeveloped eyes; damage to both copies eliminates the eyes altogether. The human version of the gene, *Aniridia*, was found because mutations in one copy cause defects in the iris, lens, cornea, and retina.

The Gehring group's discovery that *eyeless* is the fruit fly counterpart of *Aniridia* and *Small eye*, published last August in *Science* (5 August 1994, p. 785), came as a surprise, says Charles Zuker of UC San Diego, who wrote a Perspective that accompanied the paper, because the compound eyes of insects and the single-lens eyes of vertebrates are so different that biologists have generally assumed they evolved independently. "There are a dozen ways you can make image-forming eyes, just based on optics," says Zuker. "If you ask how many of those are found in evolution, you find that each and every one is." Such diversity had led researchers to conclude that eyes evolved independently 40 or more times. But the common requirement for the *eyeless* gene stood out as a striking link between such divergent eyes, suggesting that the eyes of insects and mammals didn't spring from different origins, but somewhere in the distant past shared a common ancestor.

Aside from its evolutionary implications, the finding raised in Gehring's mind a possible connection to a phenomenon he had studied 30 years ago as a graduate student with Ernst Hadorn at the University of Zurich. In fruit flies and other insects, embryonic tissues called imaginal disks give rise to adult structures such as wings, eyes, and legs. When imaginal disks are removed from fruit fly larvae and induced to develop in culture, they usually retain their identity—wing disks form wings, leg disks form legs, and so on. But Hadorn found that occasionally a disk would shift identity—a wing disk, for example, might form an eye—a process he dubbed transdetermination.

The mystery of transdetermination was never solved, but Gehring and others came to suspect that it resulted from the accidental switching-on of master genes that had the power to turn on a different developmental program. When Quiring cloned *eyeless*, Gehring had a hunch it might be one of these putative master control genes. Gehring postdoc Patrick Callaerts and graduate student Georg Halder tested that hunch by engineering flies to express the *eyeless* gene in imaginal disks destined to form wings, legs, and antennae.

"If you rounded up 10 people and asked, 'Do you think you could actually [make eyes with such an experiment],' you would probably get 10 different reasons why it wouldn't work," says Berkeley's Rubin. But Gehring would not have been among the skeptics. Recalling the transdetermination experiments of 30 years ago and the "brilliant red pigmented eye facets" that arose in his wing-disk cultures, Gehring says he believed the experiment would generate eyes.

He was right. "Flies started to hatch with huge eyes on their wings," Gehring says. And on their legs. Some even had eyes on the ends of their antennae that looked "like little crab eyes" on stalks. The eyes appeared to be

perfect copies of normal eyes. "These eyes have everything a [normal] eye has, from the bristles to the lens to the pigment cells," says UCSD's Zuker. And Gehring says his team recently confirmed that the eyes are functional as well: Their photoreceptor cells respond to light.

These results stand in stark contrast to what usually happens when developmental biologists coax a tissue to take steps down the wrong developmental path. Typically the wayward tissue may make a protein or a partial structure characteristic of the path it is forced to take—but not a whole organ. In the case of *eyeless*, says Rubin, "you are definitely not talking about the tissue just expressing an eye [protein]. You are making a whole tissue with all its complexities."

This is not, however, the first case in which genetic engineering has been used to design flies with complete structures in the wrong place. In 1987, for example, Gehring's group turned on a gene called *Antennapedia* in the heads of flies, where it is not normally expressed, and as a result the flies grew legs where their antennae should have been. But that experiment and others like it were fundamentally different from the *eyeless* experiment, says William McGinnis, who studies fruit fly development at Yale University. *Antennapedia* is not a master gene for leg formation, he says, but instead is concerned with "assigning a spatial position" to a tissue. Turning on *Antennapedia* in the head tells the head to develop as the midbody would, and that includes the formation of legs instead of antennae. In contrast, the *eyeless* gene, McGinnis says, specifies "a functional organ rather than a spatial identity."

The ability to induce a complete organ is what appears to make *eyeless* a "master control gene." And that is particularly exciting for researchers who study eye development in fruit flies, says UCLA's Zipursky. A great deal is known about the genes that cause the production of the different cell types in the eye—genes such as *sevenless*, which triggers the formation of one of a specific type of photoreceptor cell, or the genes that code for the rhodopsin pigments that detect light. But little is known about the hierarchy of regulatory genes that turn on these and the hundreds of other genes necessary to form eyes. *Eyeless* may help change that. "What's very exciting here is that *eyeless* is really high in that hierarchy," Zipursky says. Using a variety of experimental methods, researchers should be able to find the genes that are activated by *eyeless* and thus begin to fill in the complex cascade of genes turning on genes that leads to the eventual activation of the structural genes that make eyes.

The current paper also strengthens the evolutionary connection that was made when the link between *eyeless*, *Small eye*, and *Aniridia* was discovered, says Nipam Patel, a developmental biologist at the University of Chicago who studies the evolution of genes that control development. As different as the eyes of flies and mice are, says Patel, the finding that they not only share a common control gene, but that the mouse form of the gene can function in flies, is powerful evidence that they have a common ancestry.

Patel says evidence of a common ancestry in no way contradicts the view that image-forming eyes evolved independently. It merely means they diverged from a common ancestral eye and that the development of that eye was likely governed by the ancestor of the *eyeless* gene. That early eye, says Patel, may have been a mere eyespot, a cluster of light-sensitive cells with no image-forming ability that is common in lower animals. But as nature improved on that eye in all the ways that led to various image-forming eyes, it apparently continued to use the *eyeless* gene to control the ever-more complicated process of eye development.

That suggests that *eyeless* should participate in eye formation in other species as well. And that may well be true. Gehring's group has already found counterparts of *eyeless* in a diverse range of animals including squid—which have very advanced image-forming eyes—and planarians, tiny flatworms with rudimentary eyespots, although the gene has not yet been shown to be essential for eye formation in these animals.

As researchers use *eyeless* to probe the mysteries of eye formation in fruit flies and other animals, they will undoubtedly make comparisons up and down the phylogenetic tree. And the similarities and differences they find will lead to a better understanding of just how much our eyes do have in common with those of our distant animal relatives—as well as how our eyes are formed. The one thing it won't do is give us eyes in the back of our heads, useful as that might be.

–Marcia Barinaga

PARTICLE PHYSICS

Searching for the Spin of the Proton

Since its 1992 inauguration, the Hadron-Electron Ring Accelerator (HERA) at DESY, Germany's particle physics laboratory near Hamburg, has been the front-runner in the effort to understand what goes on inside protons—the particles which, along with neutrons, make up the atomic nucleus.

Toward the end of this month, researchers at DESY will start up a new $20-million detector called Hermes—a collaboration among 10 countries (Armenia, Belgium, Canada, Germany, Italy, Japan, the Netherlands, Russia, the United Kingdom, and the United States)—aimed at answering one of the most nagging unknowns in physicists' understanding of the proton: what carries its spin. Theory predicted that a proton's spin was obtained by adding together the spins of its three main components—quarks. But a shock result in 1988 showed that the constituent quarks contribute only part of the spin. "It came as a surprise," says Hermes collaboration member Richard Milner of the Massachusetts Institute of Technology (MIT). "People believed that our understanding of the spin of the proton was in good shape."

The spin of a subatomic particle is very different from the spin of a billiard ball. For a start, the spin is not created by an external force, but arises from within, and in common with many quantities described by quantum mechanics it can only assume discrete values. Quarks, the building blocks of protons and neutrons, can only have spin states of +1/2 or –1/2, which are usually symbolized as spins with their axes pointing up or down. Protons and neutrons—the nuclear constituents known as nucleons—always have a spin of +1/2.

Seven years ago, the neat picture of quark spins adding up to make the nucleon's spin was shattered by the results of a group called the European Muon Collaboration working at CERN, the European particle physics center near Geneva. Subsequent experiments at CERN and at the Stanford Linear Accelerator Center in California revealed that the traditional picture was fundamentally inadequate: Only about 30% of a nucleon's spin comes from its main quarks.

So where does the rest come from? Earlier experiments at DESY offer some clues. The HERA ring is unique in that it can accelerate counter-rotating beams of electrons and protons and smash them together to probe the proton's structure. Experiments with HERA's two existing detectors, H1 and Zeus, confirmed that the three main quarks—known as "valence" quarks—play a major role, but that the interior of the proton is also awash with other particles (*Science*, 24 June 1994, p. 1843). These other nuclear constituents include gluons, which carry the "strong" force that binds the valence quarks together, along with "virtual" quarks and antiquarks that pop up out of the vacuum in pairs and instantly recombine, annihilating each other.

Those additional findings suggested that whatever provides the missing 70% of the proton's spin is to be found in this tangle of particles. But precisely where is a mystery. According to Robert Jaffe from the Center for Theoretical Physics at MIT, all that the current experiments "tell us for sure is that the valence quarks do not carry all the spin,