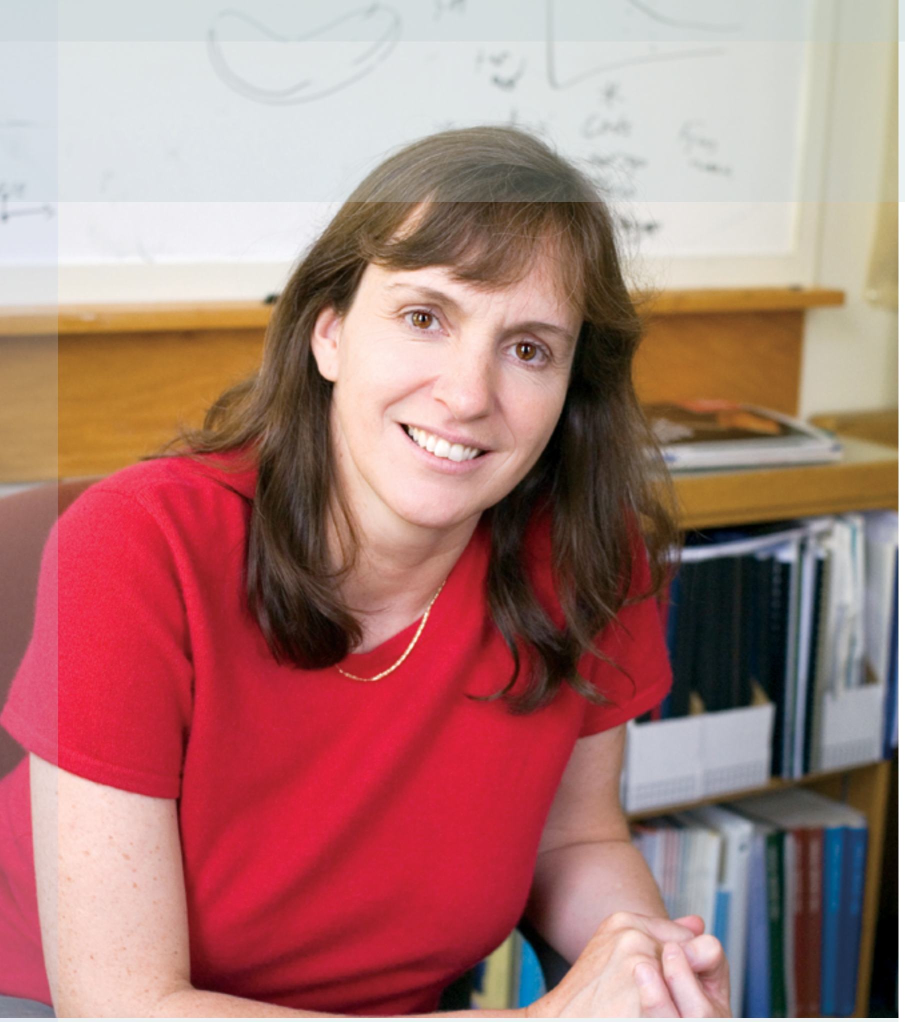


RESEARCH PROFILE

Marja Timmermans

A feeling for the organism

CSHL Professor Marja Timmermans, a distinguished plant geneticist, is solving mysteries about essential mechanisms in plant development that have been perplexing people for hundreds of years. Her most recent research explains the developmental process in which emerging plant leaves “know” how to make distinct top and bottom surfaces. The work has implications in fields as wide ranging as agriculture and human health.

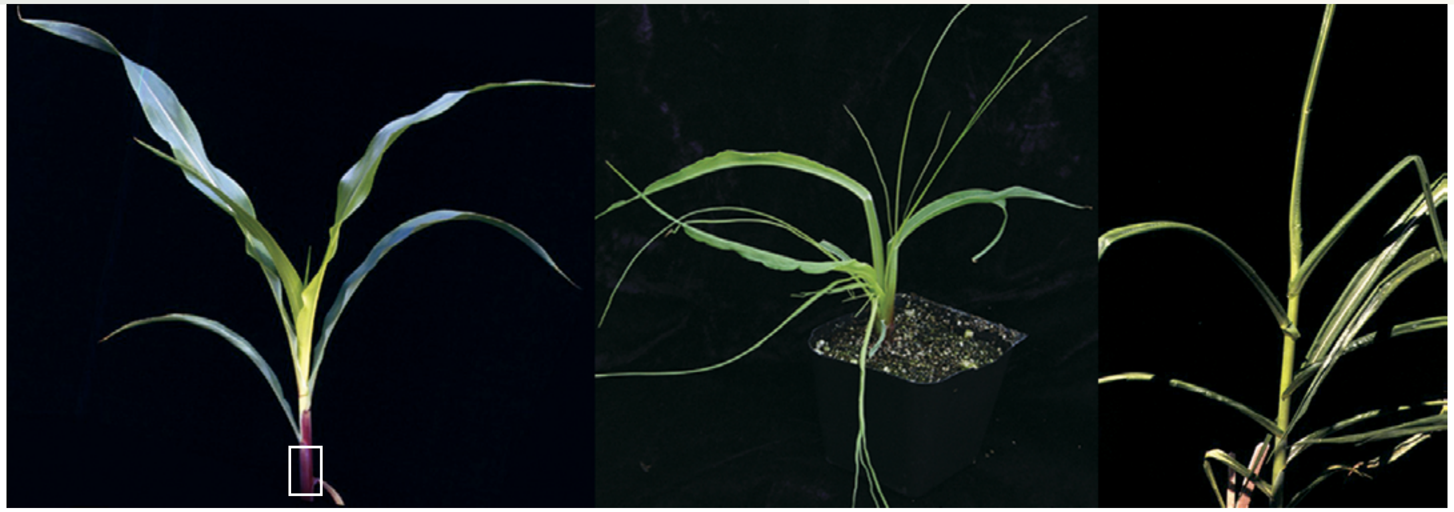
It took years of painstaking effort for Timmermans to unlock the secret of leaf polarity. The key discoveries, which include the identification of essential genes and surprising observations about signaling between cells, had to be assembled one by one, like pieces of a complicated jigsaw puzzle.

During her youth in the Netherlands, Timmermans was drawn to math and problems in logic. “It has always been puzzles that have really excited me,” she says. This explains what would otherwise be a curious fact about her early life: upon finishing high school she aimed not to be a scientist but a police detective.

In 1987, while serving as a teaching assistant for the CSHL Plant Course, Timmermans met and was deeply impressed by Barbara McClintock, the CSHL scientist who had recently won the Nobel Prize for plant genetics work performed decades earlier. A new age was dawning, and discoveries made by McClintock—of “jumping genes,” bits of DNA that hop around randomly in genomes, causing havoc—seemed to Timmermans all the more astonishing, given the primitive state of knowledge about genes when the discoveries were made.

This taught Timmermans a lesson, which she says is perfectly encapsulated in the title of a 1983 McClintock biography: *A Feeling for the Organism*. “Barbara really had a vision of the whole plant. From pigmentation patterns on the plant, she asked: what happens genetically and during development to make these patterns emerge in the form that we see?”

Timmermans went on to earn a Ph.D. in biochemistry at Rutgers University and set up her own lab at CSHL in 1998 as a CSHL Fellow. (She now directs the Fellows program.) A staff scientist beginning in 2001, Timmermans was a member of a pioneering cohort then using an approach called forward genetics to make new discoveries.



Normal maize leaf (left) is broad and blade-like. (White box: the meristem, its stem-cell reservoir.) In maize mutants identified by Timmermans, aberrant genes cause leaves to develop abnormally. In *leafbladeless1* (center) leaves are conical and thread-like; in *rolled* (right) they curl up.

Every summer and winter, she and members of her lab, working in the Uplands Farm greenhouse, would plant 20,000–30,000 seeds in plastic flats. They were trying to find genes involved in setting up the top and bottom surfaces of leaves. “The ‘forward’ method was to plant massive numbers of seeds with a predisposition for mutation. In screening for mutant seedlings—misshapen because of gene lesions—we screened for particular types of defects that we reasoned would be informative of leaf polarity.”

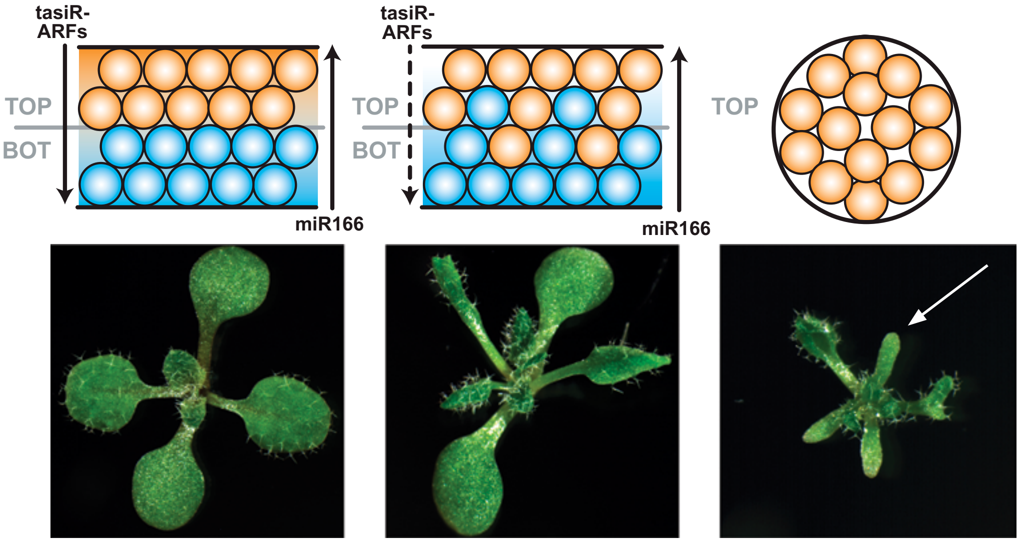
A flat leaf is marvelously evolved to perform two related but highly distinct functions. The top side is a natural solar cell: it harnesses light from the sun to generate food for the plant. The leaf’s bottom side serves as a place where gases enter and exit. To perform these dissimilar tasks, many leaves assume a flat, blade-like form.

The first plant Timmermans worked on at CSHL “was this cute maize mutant called *leafbladeless1* (*lbl1*) that came out of one of our screens.” Maize plants in which *lbl1* was mutated grew radial, thread-like leaves instead of the sturdy, broad-faced leaves of healthy maize plants. But why? What biological process was the gene a part of, and what went wrong when the gene was aberrant?

Over a period of years, forward genetics enabled Timmermans and her team to make an important discovery: the *lbl1* gene in a normal maize plant generates a small RNA molecule called tasiR-ARF, whose action is essential in establishing the identity of a developing leaf’s top surface. When *lbl1* is mutated, as in the plants Timmermans screened in the greenhouse, the leaf is unable to develop in a blade-like form.

In the early years of the 2000s Timmermans used the same method to make a second discovery: a maize gene called *rolled* that when mutated caused emerging leaves to curl up. After tracing a series of developmental steps, Timmermans demonstrated that expression of this gene was ultimately tied to the generation of another essential small RNA—a microRNA called miR166. It proved to be indispensable in establishing the bottom surface of a blade-like leaf.

The stem-cell reservoir in maize (white box, insert). Emerging leaves begin as bumps (arrow 1); successive young leaves show characteristic top-bottom patterning: a small RNA called miR166 localizes to the bottom side of each (arrows 2–4), one of two key polarity signals.



How do leaf cells know whether to be ‘top’ (orange) or ‘bottom’ (blue)? Three images of mustard plant leaves, each corresponding with a drawing, above. In normal leaf development (left) two small RNAs assemble on the leaf to form opposing signal “gradients”: tasiR-ARF is strongest at the top and miR166 strongest at the bottom. Center: “top” signal is weak, resulting in ill-defined boundary; leaf is curled. Right: “bottom” signal is missing; leaf, all “top,” is radial.

Finding two genes essential for leaf polarity was a major coup. “It was a real surprise to discover that small RNAs played a central role,” she says. But another major surprise was in store, in work that has come to fruition only in the last several years.

The geneticist as detective

Now using an approach called reverse genetics, her team begins with a gene of interest—like *lbl1* or *rolled*—and tries to pinpoint events “downstream” of its expression that go awry when the gene is mutated. “You start building these hierarchies of pathways, and you assemble a diagram: this gene…interacts with these other genes…which regulate this process, with these consequences…”

The challenge is to discover when, where and how during leaf development the expression of genes sets off a cascade of signaling events within leaf primordia. These are tiny bumps, incipient leaves just emerging from the stem-cell reservoir at the growing tips (meristems) of plants. Scientists have long known that signaling from the meristem somehow tells emerging leaves to form top and bottom sides. Forward genetics had enabled Timmermans to establish that small RNA molecules are involved; reverse genetics now led her to explain how these small RNAs give leaf cells positional information about what function to perform.

Unexpectedly, the small RNAs she had previously linked with leaf defects turned out to be mobile signals. By moving between cells, tasiR-ARF and miR166 form “concentration gradients” across the thickness of the emerging leaf [see illustration]. Based on where each cell sits along these gradients, it knows if it is going to be “top” or “bottom”—that is, whether it will develop into a light gatherer or gas exchanger.

Timmermans’ discovery that microRNAs move from cell to establish leaf polarity marked the first time that small RNAs native to an organism were shown to be capable of mobile signaling. “At first this was thought to be something quirky about plants,” says Timmermans. Recently, though, small RNAs encapsulated within tiny spheres called vesicles have been shown to signal by traveling from cell to cell in mammals. Since signaling snafus are central in many human disease processes, work on mobile signaling by Timmermans and others “could very well” have future applications in the development of new human therapeutics, she says.

There is a more proximate application of the work in agriculture. Increases in maize yield have been obtained from optimizing the way leaves are positioned on the plant. Upright, as opposed to floppy, leaves enable farmers to increase yield by planting closer together. “One of the key traits that gives you upright leaves is the polarity pathway we have been working on,” Timmermans notes. “We will want to tweak just a little bit—to make a little bit more ‘top’ than ‘bottom’.”

The detective in Timmermans is not ready to consider mobile signaling in leaves “case closed.” “We still don’t know how a particular cell actually senses the miRNA, measures its signal, and then knows how to react appropriately. It’s a tremendously complicated puzzle!”

Peter Tarr