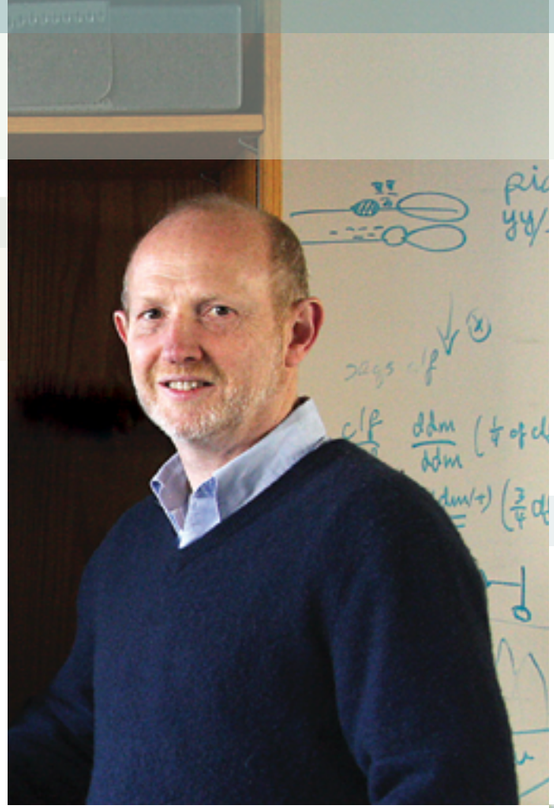
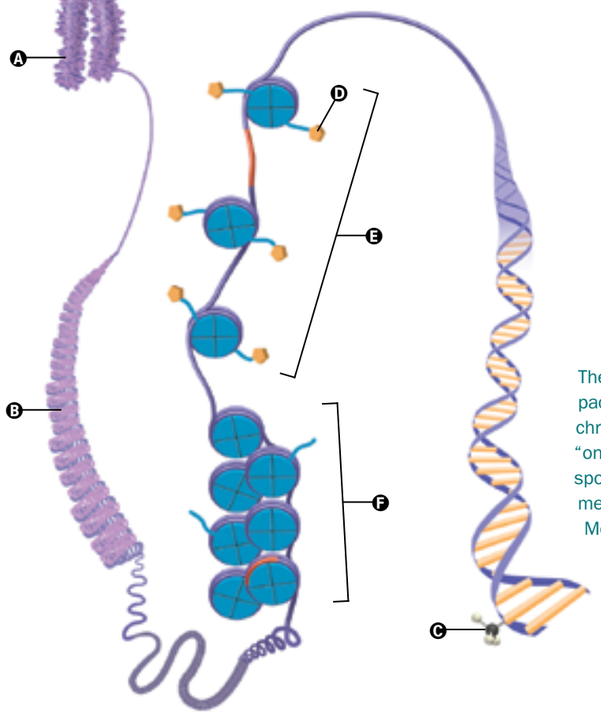
RESEARCH PROFILE

Robert Martienssen

In the 63 years since Watson and Crick discovered the double helix, we've all learned that the genome is "the blueprint for life." For the last 25 years, CSHL's Rob Martienssen, as much as any other scientist, has enabled us to understand that while this is true, having the blueprint—whether for single-celled yeast, plants or people—is only the first step in understanding what makes genes act the way they do.

Martienssen, a CSHL Professor, Fellow of the Royal Society, and Investigator of the Howard Hughes Medical Institute and Gordon and Betty Moore Foundation, is a plant geneticist who has made trailblazing discoveries in the field of epigenetics. That term, proceeding from the prefix "epi," directs our attention to a realm of action that occurs literally "on" or "on top of" the genome.





Think of the double helix as a long, twisting ladder, to which chemical tags of various kinds can attach. When a methyl group (chemical formula CH_3) binds at one of the ladder's "rungs"—*i.e.*, a DNA base—it can change the activity of the underlying gene, typically silencing it. Why? The protruding methyl group prevents specialized machines from attaching at that genome position and copying the DNA—the first step in gene expression.

Martienssen and collaborators have been among the leaders in assembling a comprehensive account of how several overlapping layers of epigenetic signals function, interacting in complex ways to regulate gene activity.

The DNA of living things is compressed into inconceivably small packages called chromatin (B), which is itself bundled to form chromosomes (A). Epigenetic signals are chemical modifications "on top of" DNA itself, or the histone proteins around which DNA is spooled to form chromatin bundles. The drawing at left shows how methyl groups (CH_3) can attach directly to the double helix (C). Methylation and other epigenetic marks, collectively called epigenetic factors, can also attach to tiny "tails" on histone proteins (D). Their presence or absence helps determine whether the DNA within the bundle is "open" and thus accessible to DNA copying machinery; or "closed" and inaccessible. Genes within DNA in open chromatin areas (E) can be expressed; in closed areas (F), they are silenced.

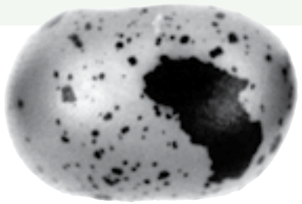
This integrated account is of great relevance in efforts to coax plants to higher yields or adapt to changing environmental conditions. It's also important in efforts to understand human diseases, from neurodegeneration to cancer. More broadly, it tells us something profound about life. Just like genetic mechanisms, epigenetic mechanisms that regulate genes are a product of evolution operating over hundreds of millions of years. Keeping genomes intact, and, in Martienssen's words, ensuring "the immortality of the germline," they are an important part of what makes life so remarkably robust and enduring.

Moving beyond Mendel's Laws

In the 1980s, as an undergrad at Cambridge in his native England, Martienssen received a classical training in genetics. To his inquiring mind, "anything that *contravened* formal genetics was interesting." Indeed, Mendel's "laws" couldn't account for plenty of things—for instance, the way a plant or animal's genes respond to the environment. Events that occur during the life of an organism, such as stress, can significantly alter gene activity and function, and these changes can be passed down to progeny.

Martienssen and McClintock in the greenhouse, *ca.* 1990.

The scientist who was Martienssen's greatest inspiration and influence did advance theories on such phenomena. Her name was Barbara McClintock, and in a way that he could not have anticipated, his discoveries have proven the existence of epigenetic mechanisms she predicted decades earlier. He had the thrill of working at McClintock's side in his first 2 years at CSHL.



McClintock linked spots on corn kernels to jumping genes.

Martienssen's earliest research extended McClintock's most famous observation. Working "in Barbara's favorite organism, maize," the newest CSHL faculty member demonstrated what happened when a chunk of DNA literally jumped into a new position in the genome. Back in the 1940s McClintock had described "jumping genes," or as scientists would later call them, transposable elements (TEs). It took 40 years for the importance of this discovery to be recognized, in the form of a Nobel Prize.

Having observed curious patterns of spots in corn kernels, McClintock had famously traced them to the impact of a TE jumping into a gene that controlled kernel color. In his first published paper, Martienssen showed how a different transposon generated similar patterns of stripes in leaves of the maize plant.

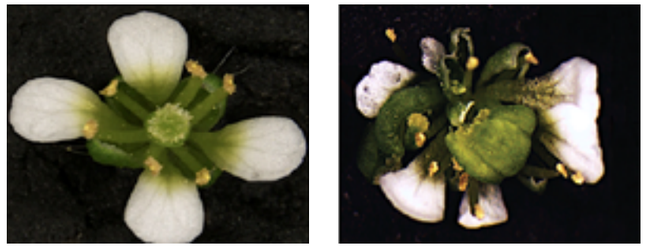
He succeeded in identifying the gene into which the transposon had jumped; more important, he discovered what was in effect an epigenetic on-off switch. When the transposon was unmethylated, the gene was silent; when methylated, the gene came back "on." This suggested something broader that Martienssen and others would flesh out more fully: *methylation keeps transposons in check*—prevents them from hopping at will from genomic place to place, interrupting genes, and inevitably, threatening the viability of the organism. Epigenetic control, it became clear, was a kind of genome defense mechanism.

In future years, Martienssen and others would discover that various epigenetic mechanisms were interrelated. His experimental approach was subtractive. "I'm a geneticist," he explains, "and so I tend to take things away or look for things that are missing and find out what goes wrong." For instance, believing methylation to be important in the suppression of TEs, he sought further evidence by finding mustard plants (*Arabidopsis thaliana*) in which methylation was notably absent. He traced this oddity to a mutated gene called *DDM1*.

DDM1 showed Martienssen something no one expected, and for a long time, few believed: that methylation and another gene-regulating process called chromatin remodeling "were actually two sides of the same coin."

Chromatin is the packaging used by cells to bundle their genomes; it consists of an organism's DNA, spooled around proteins called histones. When chromatin is very tightly bundled, the DNA it contains is inaccessible to the cell's gene-expressing machinery. Only DNA in loosely packed chromatin can be expressed. Chromatin remodeling factors help determine what genes can be expressed at a given moment in time. Martienssen's discovery that methylation can affect chromatin's form shed light on a poorly understood process.

The lab's epigenetic experiments focused on a structural location in chromosomes, the centromere, where DNA sequences were known to repeat over and over again. These "centromeric repeats" were considered "junk DNA" by some, but Martienssen, thinking of McClintock, suspected otherwise. They were a signature of TEs.



Loss of methylation in mustard plants results in massive activation of TEs; the impact is devastating.

In 2004, a Watson School graduate working in Martienssen's lab named Zachary Lippman (now a CSHL Associate Professor) was first author on a paper Martienssen considers one of his most important. By this time, another project that Martienssen had worked on with CSHL's master gene sequencer, Dick McCombie, and others, was complete. They had assembled the first complete plant genome, that of the mustard plant *Arabidopsis*.

Having the sequence in hand enabled Martienssen and Lippman to design experiments showing that areas of repeat DNA in and around centromeres and in a few other dense, knob-like regions in the *Arabidopsis* genome—composed of tightly packed chromatin—were full of TEs. Importantly, their DNA was peppered with methylation marks, accounting for their silencing. But in plants in which *DDM1* was mutated—plants that lacked methylation—the team showed how TEs sprang to life, harming genome integrity and eventually rendering the plant sterile.

This and related work validated a momentous theory McClintock had ventured decades before, without benefit of the modern tools of molecular biology. She deduced that TEs, by jumping into or next to genes, could actually shape evolution. Martienssen's group now could show a specific example, a TE that jumped next to a gene in *Arabidopsis* that controls the time to flowering. Since the TE was methylated, the timing gene was effectively *controlled* by the TE, which thus determined the plant's developmental fate. Says Martienssen: "The way in which that gene was controlled epigenetically was exactly what Barbara predicted when she called TEs 'controlling elements.' That's how she discovered epigenetics—because TEs can control genes."

This same line of research brought Martienssen's work into conjunction with that of CSHL's Greg Hannon, then investigating a recently discovered mechanism called RNA interference (RNAi). Like epigenetic mechanisms, RNAi was native to cells and could silence gene expression. In mutant *Arabidopsis* plants, and then in fission yeast, which have very simple genomes, Martienssen's team identified a protein that would later be named Argonaute—a key component of the RNAi gene silencing machinery.

In 2002, the journal *Science*, citing "electrifying discoveries" by Martienssen and several others, called advances in understanding RNAi and epigenetics the "Breakthrough of the Year." Martienssen had the satisfaction of seeing his research on methylation and chromatin remodeling converge with new experiments on RNAi in yeast. By subtracting the RNAi machinery from yeast cells, he showed that tightly packed chromatin (the TE-rich portion, which biologists call heterochromatin) could no longer form properly at the centromere of chromosomes, causing cell division to go awry.

This work revealed how small RNAs generated by repeat DNA sequences in heterochromatin are needed to guide chromatin modification. Today, says Martienssen, it's understood that "all these mechanisms—RNAi, chromatin modification, DNA methylation—help to silence heterochromatin. They depend on each other. If you take any one of them out you tend to disrupt the other two. Somehow it all works to keep the genome intact, and to prevent TEs from damaging anything."

In recent years, the lab has tackled another big mystery. With every generation there is a complete erasure of epigenetic marks in germline cells, sperm and eggs, in many plants and animals. This "starting afresh" would seem to provide a brief period during "reprogramming" for transposons to run amok. The lab's work on pollen has revealed an astonishing reprogramming trick that skirts potential disaster. Individual pollen grains consist of two cell types: emergent sperm cells and the nurse cells that support them. A nurse cell, bearing the same genome as the corresponding sperm cell, "de-represses" its TEs by removing epigenetic marks, and then makes small RNAs (called easiRNAs) to mark the vacated genome positions. These small RNAs are later taken up by the sperm cell, in which they guide methylation marks to the proper DNA sequences, ensuring that TEs in the male germline will remain silenced genome-wide.

Solving environmental problems

Martienssen is sanguine about future work in the lab. "As we learn more and more about plant biology, I am confident we can come to grips with some really big problems," he says, among them boosting global food production and slowing climate change. There are practical limits to new variation that plant breeders can generate in staple crops, he notes. One way of rising above approaching thresholds is to discover better ways to clone prized varieties, while avoiding pitfalls like those seen in oil palm [see box below]. To clone a prized plant is to reproduce it in the absence of fertilization, *i.e.*, without having it reproduce sexually—an act that inevitably involves "shuffling the genome" and wiping the genetic slate clean. The lab is engaged in adapting a process of asexual reproduction called apomixis, which occurs naturally in some species, such as dandelion. It involves tricking a prized variety of a sexually reproducing plant, say an important staple like rice or wheat, to clone its own seeds and thereby ensure that the next generation retains all of the superior characteristics of the parent.

A second forward-looking project pivots on the fact that "plants are the fundamental arbiters of climate change." As Martienssen notes, "Plants fix carbon—they take in carbon dioxide and they expel oxygen. Animals do the reverse." A first step is to produce renewable, carbon-neutral biofuels—fuels made from living rather than fossilized plants. "In fossil fuels, we take carbon that plants successfully took out of the atmosphere eons ago and reintroduce it!" The ultimate solution, however, is to grow a low-maintenance, highly adaptable, carbon-loving plant like duckweed in vast quantities, and then to sequester the plants so that the carbon they remove from the air remains fixed in perpetuity.

Peter Tarr

The secret of bad *karma*

The fruit of 25 years of research is well represented in this image of the ruined fruit of an oil palm clone, the "victim," improbably enough, of bad *karma*. In a paper that brings a career's worth of epigenetics insight to bear on a costly problem in tropical agriculture, Martienssen and colleagues showed in a 2015 *Nature* cover story that loss of methylation by a single transposable element (TE) embedded in the oil palm genome has caused the loss of hundreds of millions in ruined crops. When high-yielding trees are cloned, they're grown in culture, from detached leaves. The team traced the problem to a gene called *mantled*, "which, in a way McClintock would have appreciated, happens to be under the control of an adjacent TE," called *karma*. The result: ruined "mantled" fruit—the result of an oil palm leaf, detached from its meristem, having lost the ability to manufacture small RNAs to guide *karma* methylation. Such ill-fated clones can now be spotted in advance, saving breeders the effort of raising trees that yield worthless fruit.