During the past decade, it has become clear that epigenetic mechanisms play a particularly active role in the brain. Research by UAB neurobiology chair David Sweatt, Ph.D., for example, proved that it is impossible to form and store new memories without epigenetic tags. Epigenetic dysregulation, other researchers have shown, is involved in many neurological disorders, including Alzheimer's disease, schizophrenia, depression and addiction.

"Just about any neuronal phenomenon can be related to the central epigenetic programming of cell differentiation or cell function or information storage," said Jeremy Day, Ph.D., assistant professor in the UAB Department of Neurobiology, whose lab is investigating the effects of epigenetics in learning, memory and addiction. Now, an emerging set of molecular tools is giving scientists the ability to manipulate the epigenome in unprecedented ways. In two recent review papers, Day and UAB colleagues capture the excitement surrounding these new "precision epigenetic editing" techniques, which can add and erase epigenetic marks at specific locations throughout the genome.

Researchers are already using the tools to gain a deeper understanding of epigenetic mechanisms in health and disease. They can also contemplate experiments that sound like science fiction, such as creating -- and deleting -- memories. And discoveries made using these tools could pave the way to a "new era of epigenetic therapeutics," as Day writes in the journal Dialogues in Clinical Neurosciences. The epigenetic drugs of the future could reverse aging-related memory impairments and inherited disorders, extinguish the traumatic thoughts of post-traumatic stress disorder, and boost cognitive function.

**Control-Alter-Delete**

Epigenetic editing strategies typically utilize CRISPR (clustered, regularly interspaced, short, palindromic repeats) or TALE (transcription activator-like effector) systems. Both systems are derived from bacteria and can be programmed to home in on specific genes, carrying an epigenome-modifying enzyme as cargo. To silence a targeted gene, a researcher can deliver an enzyme such as DNA methyltransferase (DNMT). Delivering an enzyme such as histone acetyltransferase, on the other hand, can activate a targeted gene.

TALEs, a slightly older system, require the scientist to create a customized protein to act as the homing device. CRISPRs, which use a more easily generated RNA sequence as a guide, are quickly becoming the tool of choice. Until now, researchers have been limited to using drugs such as histone deacetylase inhibitors, which "block epigenetic changes throughout the genome instead of targeting one specific gene," Day said. Those globally acting drugs have allowed researchers to establish correlations between an epigenetic change and a behavior or disease, but not prove that one is directly linked with the other. With the new precision-editing tools, investigators can add and remove epigenetic tags one by one. That will allow them to make causal connections, and identify the most important epigenetic modifications in a particular condition. They can then recreate those modifications to verify their findings. "We're going from simply being able to observe changes to being able to manipulate and recapitulate those changes in a controlled way," Day said.

"Let's say you found a modification that is associated with memory," he continued. "Now you can ask, if we generate that change specifically, can we create a memory? Can we implant a memory in the brain by changing epigenetic status? No one has been able to ask those kinds of questions before." Using next-generation gene sequencing, researchers can also catalog the entire complement of epigenetic changes involved in forming a new memory, for example, or in a specific disease, Day says. "These new tools will allow us to understand which of these changes are most important."

**Making Memories with Light**

With another emerging technique, known as optoepigenetics, researchers can also control when an epigenetic change is made. "For certain research questions, you want the manipulation to be temporally precise," Day said. Epigenetic changes associated with memory formation, for instance, may occur in a matter of minutes. Previous techniques took weeks to produce a given epigenetic change . . .
Extinguishing Memories, Reversing Inherited Diseases

The "end game" of all this research, Day says, is to develop new epigenetic therapeutics. In a paper now online in the journal Annual Review of Pharmacology and Toxicology, Day, Sweatt and Andrew Kennedy, Ph.D., a postdoctoral fellow in the Department of Neurobiology, identify four areas that could be the first to benefit from epigenetic therapeutics: PTSD, depression, schizophrenia and cognitive function. In each case, growing evidence from animal and human studies points to epigenetic dysfunction as a factor in disease progression. For example, epigenetic mechanisms "contribute to the formation and persistence of fear memories" in PTSD, according to one current hypothesis, the authors write. Altering epigenetic marks could allow for "enhanced extinction of conditioned and contextual fear" and could be used in conjunction with cognitive behavioral therapy.

Epigenetic therapies have many other exciting potential applications. They could be used to silence mutated genes that produce damaging proteins, such as the huntingtin protein responsible for Huntington's disease. That same approach, in reverse, could be used to switch on previously silenced genes to treat other conditions. Although we receive copies of genes from both our parents, one of these copies, known as an allele, can be silenced by epigenetic marks during development. In a disease such as Angelman syndrome, which is associated with severe intellectual and developmental disabilities, the active, maternal allele is mutated and nonfunctional, while the paternal allele is silenced. Removing the epigenetic tags from that paternal allele should allow it to start producing the missing protein.

Day's lab is particularly interested in new therapies for addiction. "We think that epigenetic changes resulting from exposure to drugs of abuse may endure for a long time in an addicted individual," Day said. That would explain why an addict who stays clean for several years can be immediately thrown back into a pattern of abuse by exposure to a single trigger, such as a visual cue, he says. "If we can manipulate those changes, it would be a really powerful therapeutic approach."

There are still many challenges to overcome before precision epigenetic therapies reach the clinic. Identifying the correct treatment targets will require a great deal of further basic research, Day says. Investigators will also have to perfect new, human-friendly delivery methods for these therapies; the direct injections and viral vectors used with animal models aren't feasible for clinical use. Nevertheless, "it's a really exciting time," Day said. "We can now start to answer the questions that everyone has been asking, given that epigenetic changes are present in so many conditions."

Editing the Epigenome

New precision techniques allow researchers to add and erase epigenetic marks at specific genes. Because these marks are associated with a growing list of diseases, including depression and Alzheimer's, the new techniques promise to advance understanding and could point to novel treatment options . . .

The Brave New World of Epigenetic Drugs

Although they are still only in the theoretical stage, drugs that can precisely add or erase epigenetic marks could have several unique advantages over traditional therapies. In a recent paper in the journal Annual Review of Pharmacology and Toxicology, UAB neurobiologists Jeremy Day, Ph.D., Andrew Kennedy, Ph.D., and David Sweatt, Ph.D., explained these potential game-changing attributes. A "once-in-a-lifetime" pill: Many traditional drugs require continued dosing to maintain a therapeutic effect. But by taking advantage of natural self-perpetuating mechanisms that maintain epigenetic marks, a single dose of an epigenetic drug "could last a lifetime," Day said.

Multigenerational medicine

Although the topic is still a matter of hot debate, there is strong evidence that epigenetic marks can be passed down from parents to children. That means reversing an inherited trait could benefit not just the patient but his or her progeny as well. "This represents an entirely different type of pharmacodynamics," the researchers noted in their paper: "a drug effect in the absence of the organism ever having directly experienced the drug." Epigenetic drugs also have the potential for unparalleled specificity. Traditional drugs generally work by blocking receptors on the cell membrane, Day says. But there may be thousands of these for any particular cell. An epigenetic drug, on the other hand, could silence the gene that codes for that receptor, eliminating it entirely.
Duke researchers have developed a new method to precisely control when genes are turned on and active. The new technology allows researchers to turn on specific gene promoters and enhancers—pieces of the genome that control gene activity—by chemically manipulating proteins that package DNA. This web of biomolecules that supports and controls gene activity is known as the epigenome. The researchers say having the ability to steer the epigenome will help them explore the roles that particular promoters and enhancers play in cell fate or the risk for genetic disease and it could provide a new avenue for gene therapies and guiding stem cell differentiation.

The study appears online April 6 in *Nature Biotechnology*. “The epigenome is everything associated with the genome other than the actual genetic sequence, and is just as important as our DNA in determining cell function in healthy and diseased conditions,” said Charles Gersbach, assistant professor of biomedical engineering at Duke. “That becomes immediately obvious when you consider that we have over 200 cell types, and yet the DNA in each is virtually the same. The epigenome determines which genes each cell activates and to what degree.”

This genetic puppetmaster consists of DNA packaging proteins called histones and a host of chemical modifications—either to these histones or the DNA itself—that help determine whether a gene is on or off. But Gersbach’s team didn’t have to modify the genes themselves to gain some control. “Next to every gene is a DNA sequence called a promoter that controls its activity,” explained Gersbach. “But there’s also many other pieces of the genome called enhancers that aren’t next to any genes at all, and yet they play a critical role in influencing gene activity too.”

Timothy Reddy, assistant professor of biostatistics and bioinformatics at Duke, has spent the better part of a decade mapping millions of these enhancers across the human genome. There has not, however, been a good way to find out exactly what each one does. An enhancer might affect a gene next door or several genes across the genome—or maybe none at all. To activate these enhancers and see what they do, Reddy thought perhaps he could chemically alter the histones at the enhancers to turn them on. “There are already drugs that will affect enhancers across the whole genome, but that’s like scorching the earth,” said Reddy. “I wanted to develop tools to go in and modify very specific epigenetic marks in very specific places to find out what individual enhancers are doing.”

Reddy found that specificity by teaming up with Gersbach, his neighbor within Duke’s Center for Genomic and Computational Biology, who specializes in a gene-targeting system called CRISPR. Originally discovered as a natural antiviral system in bacteria, researchers have hijacked the system over the past few years and are now using it to cut and paste DNA sequences in the human genome. For this epigenome editing application, Gersbach silenced the DNA-cutting mechanism of CRISPR and used it solely as a targeting system to deliver an enzyme (acetyltransferase) to specific promoters and enhancers. “It’s like we use CRISPR to find a genetic address so that we can alter the DNA’s packaging at that specific site,” said Reddy.

Gersbach and Reddy put their artificial epigenetic agent to the test by targeting a few well-studied gene promoters and enhancers. While these histone modifications have long been associated with gene activity, it wasn’t clear if they were enough to turn genes on. And though Gersbach and Reddy had previously used other technologies to activate gene promoters, they had not successfully activated enhancers. To the duo’s great surprise, not only did the agent activate the gene promoters, it turned on the adjacent genes better than their previous methods. Equally surprising was that it worked on enhancers as well: they could turn on a gene—or even families of genes—by targeting enhancers at distant locations in the genome—something that their previous gene activators could not do.

But the real excitement from their results is an emerging ability to probe millions of potential enhancers in a way never before possible. “Some genetic diseases are straightforward—if you have a mutation within a particular gene, then you have the disease,” said Isaac Hilton, postdoctoral fellow in the Gersbach Lab and first author of the study. “But many diseases, like cancer, cardiovascular disease or neurodegenerative conditions, have a much more complex genetic component. Many different
variations in the genome sequence can affect your risk of disease, and this genetic variation can occur in these enhancers that Tim has identified, where they can change the levels of gene expression. With this technology, we can explore what exactly it is that they’re doing and how it relates to disease or response to drug therapies.” Gersbach added, “Not only can you start to answer those questions, but you might be able to use this technique for gene therapy to activate genes that have been abnormally silenced or to control the paths that stem cells take toward becoming different types of cells. These are all directions we will be pursuing in the future.”

CRISPR-Cas9: When A GMO Is Not A GMO
Science 2.0
http://www.science20.com/american_council_on_science_and_health/crisprcas9_when_a_gmo_is_not_a_gmo-158242

[1] The genome editing technique known as CRISPR-Cas9 has taken the biology world by storm. Initially it was primarily used to knock-out (literally, to make inoperative) specific genes, however, scientists have now figured out how to use the system to knock-in genes as well as edit the epigenome. These features, along with the technique’s relative simplicity and ease of use, have led to CRISPR being adapted into a wide variety of fields such as bio-agriculture.

[2] If you aren’t yet familiar with the term, you soon will be. CRISPR-Cas9 engineered crops are very close to being on the market. Yet with all of the fear and doubt promoted about biology among anti-science groups, regulators will have to determine if they are or are not a “GMO” (genetically modified organism), a question that is harder to answer than many may realize.

[3] To begin with, GMO is really not a science term, it is a regulatory definition, a legal distinction. As science literate readers know, all organisms have had their genetically modified in some fashion, for as long as we have existed and had agriculture. Legacy techniques like artificial selection (i.e. breeding) and mutagenesis do this very randomly and at the whole genome level. Viruses can alter the genome of humans and all other cellular life too in random but often meaningful ways.

[4] A study from earlier this year described how the Agrobacterium naturally altered the genome of sweet potatoes, making them edible, some 8,000 years ago. The Agrobacterium is actually so efficient at altering the genome of an organism that scientists have used it in creating many GMOs and now scientists can use it to deliver the CRISPR-Cas9 system into cells as well. However, using this method automatically requires the legal GMO designation in America, because the Cas9 gene and fragments of the Agrobacterium’s genome can end up in the final crop.

[5] But there are ways to get around this and researchers in Korea are working hard at using CRISPR-Cas9 without incurring the manufactured stigma created by environmentalists. The current way to use CRISPR-Cas9 is by coopting the Agrobacterium (or another vector) to introduce the genes for CRISPR-Cas9 and then the cell’s own machinery is used to assemble the guide RNA and the Cas9 protein. However, if the fully assembled CRISPR-Cas9 system is introduced into the cell without using the target cell’s genome or machinery the crop would fall into a regulatory gray area.

[6] This is a legal loophole but since the definition of GMO is legal and not scientific it makes sense. Using this mechanism does not necessary preclude the GMO designation. If the CRISPR-Cas9 works correctly (and usually does) the organism’s genome will be altered in a targeted way. But by using this pre-assembly technique the researchers eliminate the possibility of “how they made it” from influencing the GMO designation and the weight of the decision on the GMO designation will depend on the “what” (i.e. the intended genetic modification) they made.

[7] This would not be the first time that genetically modified crops had circumvented the GMO designation by using alternative means. Some examples of this include blue grass made with a gene gun and plums that were the offspring of GMOs. That doesn’t mean opponents of science will stop trying to confuse the issue to create fear and doubt. A clear cut example is Bt corn, which gets a gene for a bacterial insecticide. Activists are against that even as they spray toxic Bt pesticides on organic food.

[8] Other wrinkles: What if CRISPR-Cas9 is used to delete a gene, which makes the resulting plant resistant to a deadly fungus? Or if the genome is altered to introduce ancestral traits that have been lost due to the alterations from the randomness of artificial selection? It’s easy to see how examples like this do not fit the traditional or popular view of what a GMO is, and even
more reason for policy-makers to use an evidence-basis for decision-making, and not cater to press releases. What really troubles me is that researchers are now basing their projects on loopholes in regulations instead of pursuing science or a hypothesis. They are excelling at this, but you have to wonder about the extent to which scientific thought is being hindered because of an arbitrary definition that is based on fear and not science.

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**Editing humanity:**

A new technique for manipulating genes holds great promise— but rules are needed to govern its use

*The Economist* | Aug 22nd 2015

[http://www.economist.com/news/leaders/21661651-
new-technique-manipulating-genes-holds-great-promisebut-rules-are-needed-govern-its](http://www.economist.com/news/leaders/21661651-
new-technique-manipulating-genes-holds-great-promisebut-rules-are-needed-govern-its)

[1] THE genome is written in an alphabet of just four letters. Being able to read, study and compare DNA sequences for humans, and thousands of other species, has become routine. A new technology promises to make it possible to edit genetic information quickly and cheaply. This could correct terrible genetic defects that blight lives. It also heralds the distant prospect of parents building their children to order.

[2] The technology is known as CRISPR-Cas9, or just CRISPR. It involves a piece of RNA, a chemical messenger, designed to target a section of DNA; and an enzyme, called a nuclease, that can snip unwanted genes out and paste new ones in. Other ways of editing DNA exist, but CRISPR holds the promise of doing so with unprecedented simplicity, speed and precision. A dizzying range of applications has researchers turning to CRISPR to develop therapies for everything from Alzheimer’s to cancer to HIV (see article). By allowing doctors to put just the right cancer-hunting genes into a patient’s immune system, the technology could lead to new approaches to oncology. It may also accelerate the progress of gene therapy— where doctors put normal genes into the cells of people who suffer from genetic diseases such as Tay Sachs or cystic fibrosis.

[3] It will be years, perhaps even decades, before CRISPR is being used to make designer babies. But the issues raised are already the subject of fierce discussion. In April scientists in China revealed they had tried using CRISPR to edit the genomes of human embryos. Although these embryos could not develop to term, viable embryos could one day be engineered for therapeutic reasons or non-medical enhancement. That is a Rubicon some will not want to cross. Many scientists, including one of CRISP’s inventors, want a moratorium on editing “germ line” cells—those that give rise to subsequent generations. America’s National Academy of Sciences plans a conference to delve into CRISPR’s ethics. The debate is sorely needed. CRISPR is a boon, but it raises profound questions.

[4] The only way is ethics. These questions fall into two categories: practical and philosophical. The immediate barrier is practical. As well as cutting the intended DNA, CRISPR often finds targets elsewhere, too. In the laboratory that may not matter; in people it could cause grave harm. In someone with a terrible disease, the risk of collateral damage might be worth running. But for germ-line applications, where the side-effects would be felt in every cell, the bar should be high. It may take a generation to ensure that the technology is safe. Until then, couples with some genetic diseases can conceive using in-vitro fertilisation and select healthy embryos.

[5] Moreover, awash though it is with gene-sequence data, biology still has a tenuous grip on the origins of almost all the interesting and complex traits in humanity. Very few are likely to be easily enhanced with a quick cut-and-paste. There will often be trade-offs between some capabilities and others. An à la carte menu of attributes seems a long way off. Yet science makes progress—indeed, as gene sequencing shows, it sometimes does so remarkably quickly. So scientists are right to be thinking now about how best to regulate CRISPR.

[6] That means answering the philosophical questions. There are those who will oppose CRISPR because it lets humans play God. But medicine routinely intervenes in the natural order of things—saving people from infections and parasites, say. The opportunities to treat cancer, save children from genetic disease and understand diabetes offer justification to push ahead.
A harder question is whether it is ever right to edit human germ-line cells, to make changes that are inherited. This is banned in 40 countries and restricted in many others. There is no reason for a ban on research or therapeutic use: some countries, rightly, allow research on human embryos, as long as they are left over from in-vitro fertilisation and are not grown beyond 14 days; and Britain has allowed a donor to supply mitochondrial DNA at conception to spare children needless suffering, even though the change will be passed on. And CRISPR deals with the objection that germ-line changes are irrevocable: if genes can be edited out, they can also be edited back in.

A deeper quandary concerns the use of CRISPR to make discretionary tweaks to a person’s genome. There comes a point where therapy (removing genes that make breast cancer or early-onset Alzheimer’s more likely, say) shades into genetic enhancement. Some might see being short or myopic as problems that need fixing. But here, too, the right approach is to be cautiously liberal: the burden is on society to justify when and why it is wrong to edit the genome.

It is not too soon to draw on these principles to come up with rules. Some countries may have gaps in their legislation or poor enforcement, letting privately funded scientists or fertility clinics carry out unregulated CRISPR research. The conservative, painstaking approach taken by Britain’s Human Fertilisation and Embryology Authority in its decision on mitochondrial DNA is a model. Regulators must also monitor CRISPR’s use in non-human species. Changing animals’ genomes to spread desirable traits—mosquitoes that cannot transmit malaria, for example—could bring huge benefits. But the risk of unanticipated consequences means that such “gene drives” should be banned unless they can be reversed with proven countermeasures.

If CRISPR can be shown to be safe in humans, mechanisms will also be needed to grapple with consent and equality. Gene editing raises the spectre of parents making choices that are not obviously in the best interests of their children. Deaf parents may prefer their offspring to be deaf too, say; pushy parents might want to boost their children’s intelligence at all costs, even if doing so affects their personalities in other ways. And if it becomes possible to tweak genes to make children smarter, should that option really be limited to the rich?

Thinking through such issues is right. But these dilemmas should not obscure CRISPR’s benefits or obstruct its progress. The world has within its reach a tool to give people healthier, longer and better-quality lives. It should be embraced.