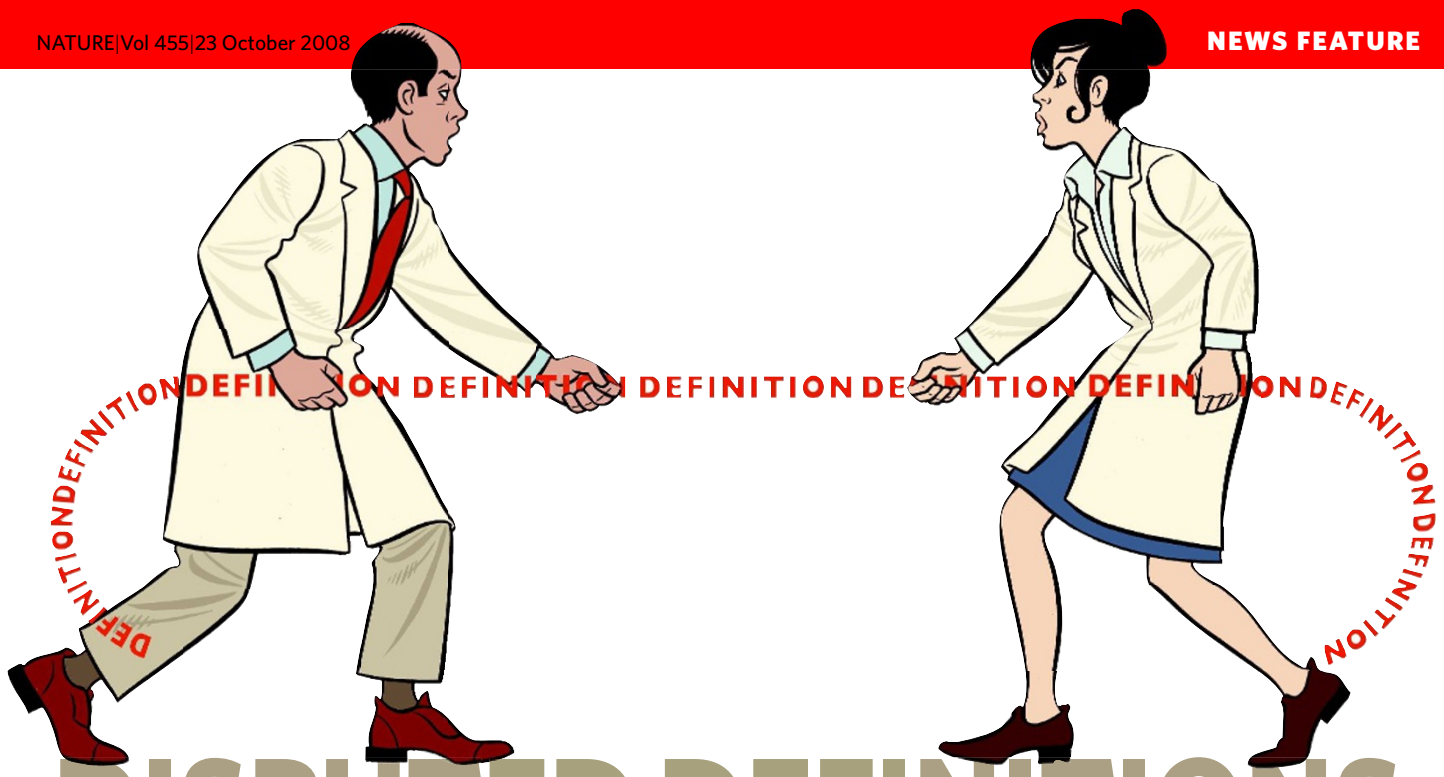


DISPUTED DEFINITIONS

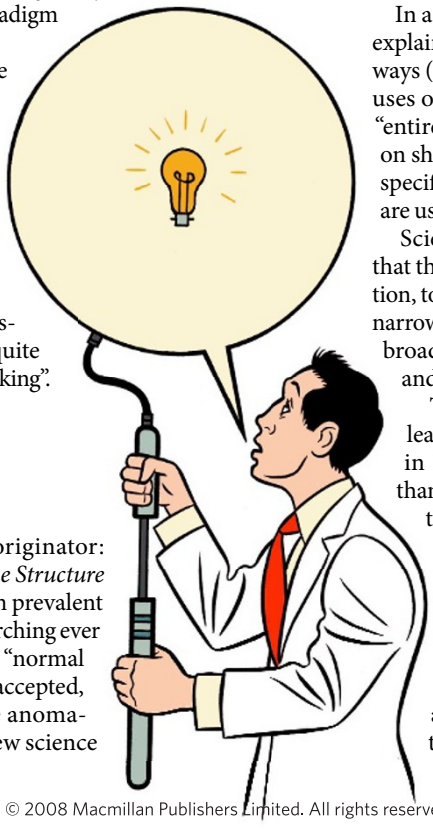
If you want to start an argument, ask the person who just said 'paradigm shift' what it really means. Or 'epigenetic'. *Nature* goes in search of the terms that get scientists most worked up.

To a great extent, science is about arriving at definitions. What is a man? What is a number? Questions such as these require substantial inquiry. But where science is supposed to be precise and measured, definitions can be frustratingly vague and variable.

Here, *Nature* looks at some of the most difficult definitions in science. Some are stipulative definitions, created by scientists for their convenience, but on which the community has not found consensus. Popular though they are, not everyone agrees on what is meant by 'paradigm shift' or 'tipping point'.

Essential definitions — those that get at the question of what makes a thing a thing — can be just as troublesome. What is race, or consciousness? And does it even matter if there is no agreed-on meaning?

The good news is that for every troublesome term there are thousands used every day with no problems. Scientists are competent, if unconscious wielders of definition, says Anil Gupta, a philosopher of science at the University of Pittsburgh in Pennsylvania, "just as one can walk quite happily without having a complete account of walking".



Paradigm shift

[ˈparɘdʌɪm ʃɪft] *noun.*

Paradigm shift has a definite origin and originator: Thomas Kuhn, writing in his 1962 book *The Structure of Scientific Revolutions*, argued against the then prevalent view of science as an incremental endeavour marching ever truthwards. Instead, said Kuhn, most science is "normal science", which fills in the details of a generally accepted, shared conceptual framework. Troublesome anomalies build up, however, and eventually some new science comes along and overturns the previous consensus. Voilà, a paradigm shift. The classic example, Kuhn said, is the Copernican revolution, in which Ptolemaic theory was swept away by putting the Sun at the centre of the Solar System. Post-shift, all previous observations had to be reinterpreted.

Kuhn's theory about how science works was arguably a paradigm shift of its own, by changing the way that academics think about science. And scientists have been using the phrase ever since.

In a postscript to the second edition of his book, Kuhn explained that he used the word 'paradigm' in at least two ways (noting that one "sympathetic reader" had found 22 uses of the term). In its broad form, it encompasses the "entire constellation of beliefs, values, techniques and so on shared by the members of a given community". More specifically it refers to "the concrete puzzle-solutions" that are used as models for normal science post-shift.

Scientists who use the term today don't usually mean that their field has undergone a Copernican-scale revolution, to the undying annoyance of many who hew to Kuhn's narrower definition. But their usage might qualify under his broader one. And so usage becomes a matter of opinion and, perhaps, vanity.

The use of the term in titles and abstracts of leading journals jumped from 30 papers in 1991 to 124 in 1998, yet very few of these papers garnered more than 10 citations apiece[1]. Several scientists contacted for this article who had used paradigm shift said that, in retrospect, they were having second thoughts. In 2002, Stuart Calderwood, an oncologist at Harvard Medical School in Boston, Massachusetts, used it to describe the discovery that 'heat shock proteins', crucial to cell survival, could work outside the cell as well as in[2]. "If you work in a field for a long time and everything changes, it does seem like a revolution," he says. But now he says he may have misused

ILLUSTRATIONS: N. DEWAR

the phrase because the discovery was adding to, rather than overturning, previous knowledge in the field.

Arvid Carlsson, of the University of Gothenburg in Sweden stands by his use of the phrase. "Until a certain time, the paradigm was that cells communicate almost entirely by electrical signals," says Carlsson. "In the 1960s and '70s, this changed. They do so predominantly by chemical signals. In my opinion, this is dramatic enough to deserve the term paradigm shift." Few would disagree: base assumptions were overturned in this case, and Carlsson's own work on the chemical neurotransmitter dopamine (which was instrumental in this particular shift) earned him the 2000 Nobel Prize in Physiology or Medicine

Unless a Nobel prize is in the offing, it might be wise for scientists to adopt the caution of contemporary historians of science and think twice before using a phrase with a complex meaning and a whiff of self promotion. "Scientists all want to be the scientists that generate a new revolution," says Kuhn's biographer, Alexander Bird, a philosopher at the University of Bristol, UK. "But if Kuhn is right, most science is normal science and most people can't perform that role."

Emma Marris

Epigenetic

[ɛpɪdʒɪnɛtɪk] *adjective.*

No one denies that epigenetics is fashionable: its usage in PubMed papers increased by more than tenfold between 1997 and 2007. And few deny that epigenetics is important. What they do disagree on is what it is.

"The idea is that there is a clear meaning and that it's being violated by people like me who use it more loosely," says Adrian Bird at the University of Edinburgh, UK . Last year he suggested this as a definition: "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states"[3]. But this wide-ranging proposal, which takes on-board pretty much every physical indicator of a gene's activity is "preposterously dumb", says Mark Ptashne of Memorial Sloan–Kettering Cancer Center in New York, who has published his own take on the word's usage[4]. "I've grown to be very careful about using the term," says Bing Ren, who studies gene regulation at the University of California, San Diego.

'So big we don't know where to start' definitions

- Science
- Life
- Natural
- Intelligence
- Ethical
- Sustainability

NOAA GOES-7/NASA

"Epigenetics is a useful word if you don't know what's going on — if you do, you use something else."
— Adrian Bird

According to the 'traditional' definition that Ptashne favours, epigenetics describes "a change in the state of expression of a gene that does not involve a mutation, but that is nevertheless inherited in the absence of the signal or event that initiated the change". The classic example is found in a bacteriophage called Lambda, which stays dormant in the genome of generations of cells through inheritance of a regulatory protein. These sort of processes are basic to some of the most pressing questions in biology today: such mechanisms are needed to explain how a single-celled embryo can generate cells that are genetically identical, but express a different array of genes and hence take on different jobs in blood, brain or muscle for generation after generation.

Over the past few years, however, all kinds of processes associated with gene control have been subsumed under the moniker. For example, 'epigenetic' is often used to refer to the chemical modification of histones — proteins that DNA winds around — which is involved in gene regulation. This infuriates those who learned the classical definition; they say it puts these modifications at the heart of development and disease despite scant evidence that they are inherited. "Why did histone marks become epigenetic?" says Kevin Struhl at Harvard Medical School in Boston. "People decided that if they call them that it makes them interesting." Others say that it is not about making things sound important, it is more the lack of any other phrase with which to collectively refer to this type of work.

The word had dual meanings long before the current debate. In the 1940s, Conrad Waddington used it to describe how the genetic information in a 'genotype' manifests itself as a set of characteristics, or 'phenotype'. In 1958, David Nanney at the University of Michigan, Ann Arbor, borrowed the term to describe "messy" inherited phenomena that could not be explained by conventional genetics[5]. "It was controversial in 1958 and everything died down and it has come alive again," says Nanney. "It took 40 years for epigenetics to become a major word in the vocabulary of modern biology."

A lot of money can ride on whether a researcher is, or is not, studying epigenetics: the US National Institutes of Health (NIH) this month started handing out US$190 million as part of its epigenomics initiative and other countries are pouring funding into the area. (The NIH is careful to define the epigenetics it is paying for as including both heritable and non-heritable changes in gene activity, something that Ptashne describes as "a complete joke".) Bird says he remains in favour of a relaxed usage. "Epigenetics is a useful word if you don't know what's going on — if you do, you use something else," he says.

Helen Pearson

Complexity

[kɔm'plɛksɪti] *noun.*

In his book *Programming the Universe*, engineer Seth Lloyd of the Massachusetts Institute of Technology in Cambridge describes how he once compiled 42 definitions of complexity — none of which encompasses everything people mean by that word. Researchers in the many institutes and programmes formed to study 'complexity' are still searching for the right way to describe their discipline. "If we're a university centre, we should be able to say what we care about," says Carl Simon, director of the Center for the Study of Complex Systems at the University of Michigan.

The quest for a rigorous definition reached a particularly intense pitch in the early 1990s, when some of the more visionary researchers at the Santa Fe Institute in New Mexico held out the hope of a universal theory of complexity — a mathematically precise set of equations that would hold for all complex systems in much the same way that the second law of thermodynamics holds for all physical systems.

James Crutchfield, head of the Complexity Sciences Center at the University of California, Davis, says that this created a problem. "New people would come into the field and start using the word 'complexity' as if it was a unitary thing" — which, as became increasingly clear, it was not. No all-encompassing theory emerged. Even within the precise world of binary code and bit strings, there was computational complexity, which describes how much memory and processing is required to carry out a calculation; algorithmic complexity, which is related to how much a digital description of something can be compressed; and any number of combinations and variations. "So my bottom line is, add an adjective to 'complexity'," Crutchfield says.

Researchers have found plenty of undeniably complex systems to study, such as economies, ecosystems, urban traffic and brains (see 'Consciousness'). And in a qualitative sense, at least, these systems do have certain features in common that might serve as a definition. They are, for instance, all composed of many independent 'agents' (consumers, species, vehicles, neurons) that are constantly interacting with, and adapting to, one another. They all display a rich array of nonlinear feedback loops among the agents, which means that small changes can have a big effect. And they never quite settle down into static equilibrium.

The effort to understand complex systems has led researchers to develop new analytical tools such as network theory, agent-based modelling and genetic algorithms. These tools, combined with the exponential growth in computational power, have allowed researchers to build ever more complex models of complex systems — and study the subtle but powerful phenomenon of 'emergence,' in which multiple agents exhibit collective behaviour that is a great deal more than the sum of its parts.

So even though the field seems little closer to defining its subject, says Lloyd, "in places where people can apply these conceptual and computational tools, we've made huge progress in understanding complex systems". But in a world where we are constructing ever more complex artefacts — technologies, economies, organizations and societies — even better tools are needed to keep pace.

M. Mitchell Waldrop

'There is a difference, honest' definitions

Molecular electronics	versus	Molecule-based electronics
Thermohaline circulation	versus	Meridional overturning circulation
Commensalism	versus	Mutualism

G. HOLLAND/PHOTOLIBRARY.COM

Race

[reɪs] *noun.*

If biologists had a list of four-letter words to avoid, then 'race' would be higher up than anything more conventionally vulgar. It is controversial, it lacks a clear definition and the more that genetics reveals about race, the more biologically meaningless the term seems.

Race was long used to imply a shared, distinct ancestry, as in a 1936 definition of the term in *Nature*: "It has two main connotations, one being community of descent, the other distinctness from other races." But in 1972, Harvard geneticist Richard Lewontin showed that the concept of race starts to dissolve under genetic scrutiny. He found that the vast majority of human genetic variation, which he measured in 16 genes, is found within, not between, what he called the 'classical racial groupings'[6]. Since then, studies examining hundreds or even thousands of genetic markers have confirmed Lewontin's findings[7,8].

A consensus now exists across the social and biological sciences: regardless of appearance or heritage, groups of human beings are overwhelmingly more genetically similar to each other than different. This doesn't mean race does not exist or is meaningless in society — far from it. But it does mean that an individual's race is not a particularly useful or predictive indicator of biological traits or medical vulnerabilities. Race is "the social interpretation of how we look, in a race-conscious society", says Camara Phyllis Jones, the research director on the Social Determinants of Health and Equity programme at the US Centers for Disease

Control and Prevention in Atlanta, Georgia. Lewontin says that assigned races are essentially arbitrary. "It means essentially a group of related people, and where you draw the line depends on where you are in history."

Some argue that severing biology from the definition of race risks jettisoning medically meaningful information. Patterns of genetic variation can be used to classify people from different geographical regions into clusters that sometimes mimic the classical racial groupings, and geneticists say that members of these groups seem to have distinctive disease prevalences and drug metabolism. So race could serve as a cheap, albeit imperfect, surrogate for defining groups for clinical trials or medical interventions.

But genetics is turning up ever more examples of how race obscures relevant information. A study published in April showed that a mutation found in 40% of African Americans acts like an endogenous beta blocker to protect patients with heart failure from death[9]. It also suggested why previous research had found conflicting evidence about the response of African Americans to beta blockers: those studies had lumped all African Americans into one group, obscuring the effects of mutations that confer protection or vulnerability.

A person's perception of his or her race can still serve to capture life experiences relevant to behavioural and clinical research, such as the stress of lifelong discrimination that may contribute to health disparities. But in other contexts researchers are abandoning the term in favour of other ways to group humans, by 'population,' genetic ancestry' or 'geographic ancestry'.

Erika Check Hayden

Makeovers

Before	After
Nuclear magnetic resonance	Magnetic resonance imaging
Clinical research	Translational medicine
Cloning	Somatic cell nuclear transfer
Genetic engineering	Synthetic biology
Lots of [genes; transcriptions; citations; etc]	[gen-; transcripto-; biblio-; etc-]omics

BLUESTONE/SPL

Tipping point

[tɪpɪŋ pɔɪnt] *noun.*

In July 2006, scientists running the RealClimate blog ironically headlined one of their posts 'Runaway tipping points of no return'. The post laments that usage of the phrase 'tipping point' in climate-change and ecosystem discussions had reached, well — a tipping point.

It's not the frequency of the word that bothers researchers. It's the lack of one clear definition and the confusing way in which the concept is being used, among scientists and in the public discourse, often to imply that global warming-induced changes will propel Earth into irreversible and catastrophic climate change. "There is no convincing theoretical argument or model that at some point the planet as a whole will snap into a second state of system," says Timothy Lenton, an Earth scientist at the University of East Anglia, UK.

The term was originally coined in 1958 by sociologist Morton Grodzins in the context of studies on the racial makeup of US neighbourhoods. He found that when the migration of African-Americans into traditionally white neighbourhoods had reached a certain level, whites began to move out. In the 1970s, epidemiologists adopted tipping point to describe the threshold at which, mathematically, an infectious disease's 'reproductive rate' goes above one. This means that each infected person infects more than one other and the disease starts growing into an epidemic.

The phrase reached its own tipping point in 2000 when Malcolm Gladwell, a staff writer at *The New Yorker*, published his successful book *The Tipping Point: How Little Things Can Make a Big Difference*. It also acquired a worrisome — some say alarmist — flavour courtesy of its frequent usage in the context of climate change.

Regarding climate, the term is commonly defined as the critical threshold at which a slow gradual change qualitatively alters the state of an entire system. This is different to a 'point of no return' which is, by definition, irreversible. Only if internal forcing will cause a runaway effect is a tipping point also a point of no return.

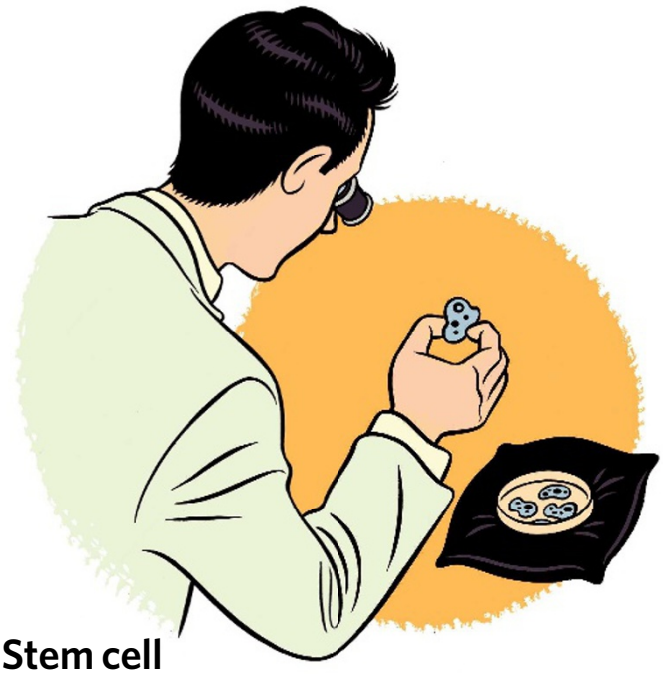
The idea that positive feedbacks — such as the melting of polar ice reducing surface reflectivity, thereby causing yet more solar absorption, warming and melting — could amplify climate change to a point of fundamentally altering the global system has been around for decades. The debate now is about where those tipping points lie, and what will happen when they are crossed.

In a paper published in February, a team led by Lenton looked at 15 potential tipping 'elements' (things that could reach tipping points) in Earth's climate system[10]. Arctic sea-ice and the Greenland ice sheet were those most at risk from 'tipping' within the twenty-first century, the authors concluded.

But researchers accept that most known tipping points seem to be reversible on human timescales. Melting of the complete Arctic summer ice sheet, for example, could probably be reversed within a few years or so in a cooler world. Melting of the extremely thick Greenland and Antarctic ice sheets are a possible exception because, once melted, new ice would have to form at lower, warmer altitudes with less snowfall.

Claims that global warming could reach an irreversible tipping point by 2016, as made last year by James Hansen, director of NASA's Goddard Institute for Space Studies in New York, refer to the trajectory of greenhouse-gas emissions, not to changes in the climate system. Even if greenhouse-gas concentrations reach a point at which they cannot be restored to pre-industrial levels, it will not inevitably push the world's climate over a catastrophic tipping point.

Quirin Schiermeier



Stem cell

[stɛm sɛl] *noun.*

Ask a group of stem-cell biologists to define stem cell, and they'll say roughly the same thing: a cell that can, long term, divide to make more copies of itself as well as cells with more specialized identities. Ask the same scientists to list the most disputed terms in the field, however, and 'stem cell' will be top of that list.

The problem here is an operational one: reasonable people disagree on which cells qualify under the definition. "It's not unusual to pick up a paper and see someone call something a stem cell and the evidence that it is, is just not there," says Lawrence Goldstein, who directs the stem-cell research programme at the University of California, San Diego.

Alleged 'stem cells' can fail to meet the definition on many counts. Stem cells should persist long term, yet many 'stem cells' exist only in the fetus. Multipotency — the ability to generate multiple cell types — is a criterion for a haematopoietic, or blood-forming, stem cell, but spermatagonial stem cells only produce sperm. Stem cells specific to tissue such as cartilage, the kidney and the cornea have been reported, with varying degrees of acceptance. The quest for a 'stemness signature', a collection of markers common to all stem cells, has been met with frustration.

Debate erupts most commonly over whether a particular cell should be considered a stem cell, which can divide indefinitely, or a progenitor cell, which can differentiate into fewer cell types and is thought to burn itself out after a certain number of divisions.

The only way to be really sure of what a cell can, and cannot do, is to observe it, but it is difficult to study cells *in vivo*, and putting them in a dish might change their behaviour. Haematopoietic stem cells were the first to be identified and have, to some extent, set default standards. Putative stem cells are isolated, then placed into animals whose own haematopoietic stem cells have been destroyed by radiation. If the blood-forming system is restored, the transplant is assumed to have contained stem cells. But such an assay is impossible when working with other cell types, such as neural stem cells, which are harder to transplant and assess in disease models. And it is difficult to pin the label to one cell type, when studies commonly involve a mixed population. "It is perhaps not realistic to come up with a generally applicable definition of an adult stem cell," says Thomas Graf of the Centre for Genomic Regulation in Barcelona.

Some researchers are side-stepping the debate by referring in their papers to 'stem/progenitor cells'. Fully understanding what each cell can do is more important than knowing what to call them all, says Goldstein. "Some of this just breaks down," he says. "That's biology. It wasn't designed to fit the language."

Monya Baker

"Some of this just breaks down. That's biology. It wasn't designed to fit the language."
— Lawrence Goldstein

Don't get us started

- Planet
- Species
- Fitness
- *Nature*

F. SAURER/SPL

Significant

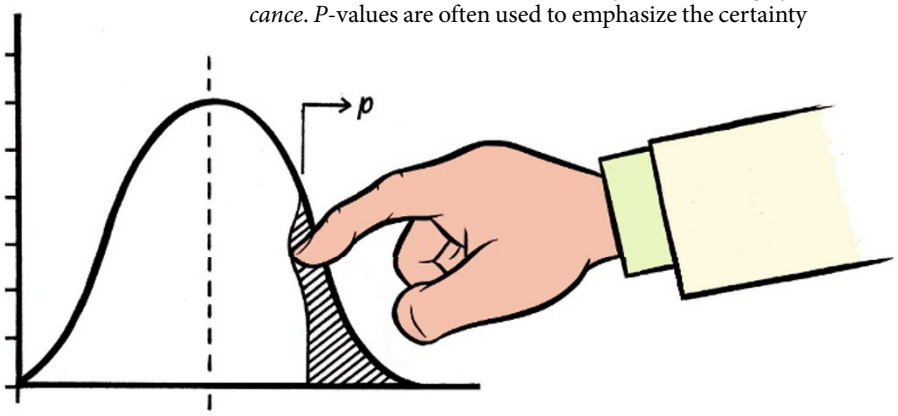
[sɪgˈnɪfɪk(ə)nt] *adjective.*

Few words in the scientific lexicon are as confusing, or as loaded, as 'significant'. Statisticians wring their hands over its cavalier use to describe scientific validity. And backed by statistics or not, researchers commonly employ the word to illustrate the importance of their latest finding.

The very definition of statistical significance is misunderstood by most scientists, says Steven Goodman, a biostatistician at the Johns Hopkins School of Medicine in Baltimore, Maryland, and associate editor on *Annals of Internal Medicine*. Typically, researchers take a result to be statistically significant based on '*p*-values'. This parameter is used, for example, to reveal whether a drug lowers cholesterol based on promising data collected in a clinical trial.

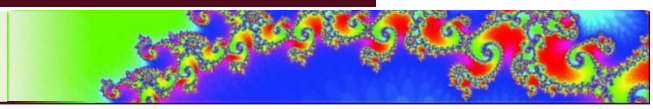
According to the common interpretation, a 'significant' result with a *p*-value of 0.05 or less means that there is a 5% or less chance that the drug is ineffective. According to the statistically accurate definition, there is a 5% or less chance of seeing the observed data even though the drug is, indeed, ineffective. Rhetorically, the difference may seem imperceptible; mathematically, say statisticians, it is crucial. In situations in which the data is somewhat ambiguous, there is a chance that results can be misinterpreted. "It's diabolically tricky," Goodman says.

Most statisticians resign themselves to abuse of the term's strict definition. But more grievous trespasses abound. "Statistical significance is neither a necessary nor a sufficient condition for proving a scientific result," says Stephen Ziliak, an economist at Roosevelt University in Chicago, Illinois, and co-author of *The Cult of Statistical Significance*. *P*-values are often used to emphasize the certainty



Yes, they do have scientific meanings: no, we don't expect anyone to listen

- Quantum leap
- Organic
- Chaos



of data, but they are only a passive read-out of a statistical test and do not take into account how well an experiment was designed. A *p*-value would not reveal, for example, that everyone was taking different doses of that cholesterol drug. In many experiments, Ziliak says, "there are so many different errors that they tend to swamp the *p*-value errors".

Even if a result is a genuinely statistically significant one, it can be virtually meaningless in the real world. A new cancer treatment may 'significantly' extend life by a month, but many terminally ill patients would not consider that outcome significant. A scientific finding may be 'significant' without having any major impact on a field; conversely, the significance of a discovery might not become apparent until years after it is made. "One has to reserve for history the judgement of whether something is significant with a capital S," says Steven Block, a biophysicist at Stanford University in California.

In some situations other statistical methods can substitute, but Goodman believes that trying to use them in the scientific literature would be like "talking Swahili in Louisiana". He says he and other editors do their best to keep the term out of *Annals* though. "We ask them to use words like 'statistically detectable' or 'statistically discernable,'" he says.

Geoff Brumfiel

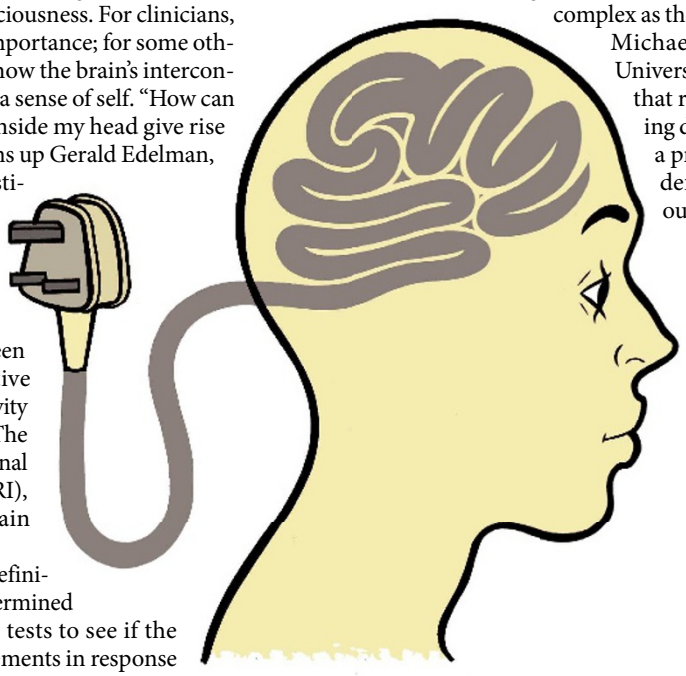
"You don't waste your time defining the thing. You just go out there and study it."
— Michael Gazzaniga

Consciousness

[ˈkɒnʃəsnɪs] *noun.*

Psychologists, philosophers, neurobiologists and doctors all grapple with the term consciousness. For clinicians, the definition is of life or death importance; for some others, it is a matter of determining how the brain's interconnecting tissues collectively create a sense of self. "How can this three-pound piece of meat inside my head give rise to something like being me?" sums up Gerald Edelman, director of the Neurosciences Institute in La Jolla, California.

In 2006, neuroscientist Adrian Owen, at the Medical Research Council Cognition and Brain Sciences Unit in Cambridge, UK, reported that a woman who had been diagnosed as being in a vegetative state had shown signs of brain activity associated with consciousness[11]. The activity was picked up with functional magnetic resonance imaging (fMRI), which can reveal changes in brain blood flow.



The finding rattled the clinical definition of consciousness, which is determined by using a series of behavioural tests to see if the patient can make voluntary movements in response to commands. The outcome can determine whether a patient needs pain medication, or whether it is time to switch off life support, but clinicians readily acknowledge that the tests break down when patients are unable to move. Doctors now find themselves in an uncomfortable limbo, because it is not clear whether cortical activity measured on fMRI is enough to redefine those decision points. "What do we do as a community as long as this method is not yet validated?" asks Steven Laureys, a neurologist with the Coma Science Group at the University of Liége in Belgium.

The French philosopher René Descartes declared that consciousness was a fundamental property that fell beyond the rules of the physical world. Most scientists, says Edelman, are not satisfied with that answer. "There must be some physical basis for consciousness," he says. "The difficulty is, how does that arise?"

Philosophers David Chalmers of the Australian National University in Canberra, explored what he called the "hard problem" of consciousness by pondering 'qualia', the subjective properties of experiences. Scientists and philosophers alike have struggled to explain how the physical properties of the world around us — such as colour and temperature — give rise to the experiences of 'red', or 'warm'. Chalmers has argued that the functional organization of the brain rather than its chemical or molecular properties makes these experiences possible.

Many definitions of consciousness include the ability to sort through the relentless onslaught of incoming data to create and respond to an internal model of the external world. And some believe that simply gathering data about neurons and behaviours will not be enough. "What we need is a 'theory of consciousness'. Then we'll be in a better position to define it," says professor of biology and engineering Christof Koch of the California Institute of Technology in Pasadena. Koch thinks that information theory could provide the solution by determining whether consciousness might be an inherent by-product of a system as enormously complex as the brain (see 'Complexity').

Michael Gazzaniga, a neuroscientist at the University of California, Santa Barbara, argues that researchers need only develop a working definition to explore consciousness, not a precise one. "You don't waste your time defining the thing," he says. "You just go out there and study it." ■

Heidi Ledford

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See Essay, page 1040.

Discuss definitions online at http://tinyurl.com/4afapl.

What Do You Mean, "Epigenetic"?

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ABSTRACT Interest in the field of epigenetics has increased rapidly over the last decade, with the term becoming more identifiable in biomedical research, scientific fields outside of the molecular sciences, such as ecology and physiology, and even mainstream culture. It has become increasingly clear, however, that different investigators ascribe different definitions to the term. Some employ epigenetics to explain changes in gene expression, others use it to refer to transgenerational effects and/or inherited expression states. This disagreement on a clear definition has made communication difficult, synthesis of epigenetic research across fields nearly impossible, and has in many ways biased methodologies and interpretations. This article discusses the history behind the multitude of definitions that have been employed since the conception of epigenetics, analyzes the components of these definitions, and offers solutions for clarifying the field and mitigating the problems that have arisen due to these definitional ambiguities.

KEYWORDS transgenerational; maternal effects; gene expression; epigenetic inheritance

INTEREST in epigenetics, as well as the usage of the term *epigenetic*, has increased significantly since the field was first conceived by Conrad Waddington in the early 1940s. In 2006, over 2500 articles related to epigenetics were published (Bird 2007), and in 2010, over 13,000 (Haig 2012). In 2013, however, this number rose to over 17,000, a striking 45 new publications every day, in addition to increases in scientific meetings and grant directives dedicated to the subject. Today, epigenetic concepts have spread into fields that do not routinely address genetics (at least explicitly), such as ecology (Bossdorf *et al.* 2008; Zucchi *et al.* 2013; Burris and Baccarelli 2014), physiology (Ho and Burggren 2010), and psychology (Ngun and Vilain 2014; Zhou *et al.* 2014). Despite its apparent popularity, the unfortunate fact is that the increased use of the term *epigenetics* is likely due more to inconsistencies in its definition than to a consensus of interest among scientists or a paradigm shift in the rules of inheritance. The term has taken on multiple meanings, describing vastly different phenomena. As a result, its usage oftentimes implies mechanistic connections between unrelated cases. The lack of a clear definition has led to confusion and misuse of the term, while also making research within the field of epigenetics difficult to synthesize and reconcile. There are many reasons why the etymology of epigenetics is so ambiguous, many of which relate to the scientific atmosphere in which the term was conceived; others are entirely philosophical. In this essay, we address these issues by providing a brief history of epigenetics (the term and the scientific field) and discussing various definitions, as well as the important differences between them. We will also address the challenges that exist, and will continue to exist, if these ambiguities are not addressed, and offer potential solutions for dealing with these challenges.

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History of the Term "Epigenetic"

To understand the meaning of the term *epigenetics*, one must understand the context in which it was derived. Conrad Waddington, who first defined the field in 1942(a), worked as an embryologist and developmental biologist. In 1947, he founded and led the first genetics department at the Institute of Edinburgh and would later found the Epigenetics Research Group in 1965 (Van Speybroeck 2002). Waddington had a strong appreciation for genetics and was an important advocate for uniting genetic principles with other fields of biology, such as cytology, embryology, and evolutionary biology; however, he was particularly interested in embryology and developmental genetics, specifically the mechanisms that controlled cellular differentiation. At the time, there were two prevailing views on development, both of which were derived from the 17th century: preformation, which asserted that all adult characters were present in the embryo and needed simply to grow or unfold, and epigenesis, which posited that new tissues were created from successive interactions between the constituents of the embryo (Waddington 1956; Van Speybroeck 2002). Waddington believed that both preformation and

epigenesis could be complementary, with preformation representing the static nature of the gene and epigenesis representing the dynamic nature of gene expression (Waddington 1956; Van Speybroeck 2002). It is through the combination of these concepts that he coined the term *epigenetics*, which he referred to as, "the branch of biology that studies the causal interactions between genes and their products which bring the phenotype into being" (Waddington 1942a; Dupont *et al.* 2009).

It is important to note that genetics was still a young field at this time, centered on Mendel's work on trait inheritance, with the *gene* being accepted as the unit of inheritance (Johannsen 1909); but, little was known about the biochemical nature of the gene or how it functioned. It wasn't until Beadle and Tatum (1941) published their work affirming the one-gene, one-enzyme concept that an understanding of gene function took discrete shape, and subsequent work on molecular biology defined gene structure. This gene-centric atmosphere, coupled with the emerging effort to understand gene regulation and expression, had a strong influence on the creation of epigenetics, both as a concept and a field of study (Jablonka and Lamb 2002).

At that time, many, including Waddington, were interested in the process of gene control and expression. Experimental embryologists, such as Wilhelm Roux (1888), Hans Spemann (1967), Viktor Hamburger (1960), and the developmental geneticist Ernst Hadorn (1955) studied mutations by inducing changes in development through experimentation with chemicals or excision. Waddington, on the other hand, was more interested in the cellular processes that brought about these changes, rather than the stimuli that created them. One of Waddington's most important contributions was his acknowledgment of, and emphasis on, the flexible relationship between genotype and phenotype (Waddington 1942a,b, 1957), and this was an idea that many of his contemporaries, such as Nanney (1958a), Huxley (1956), Ephrussi (1953, 1958), and Lederberg (1958) (see below), were also interested in. Today, Waddington's views on epigenetics are most closely associated with phenotypic plasticity, which is the ability of a gene to produce multiple phenotypes, but he also coined the term *canalization* to refer to the inherent stability of certain phenoytpes (particularly developmental traits) across different genotypes and environments (Waddington 1942b; Siegal and Bergman 2002). Together, his concepts of plasticity and canalization suggest a general decoupling of genotype and phenotype and imply that regulatory processes must exist between the two. This realization was fundamental to Waddington's concept of epigenetics.

In 1958, 16 years after Waddington first coined the term, David Nanney published a paper in which he used the term epigenetics to distinguish between different types of cellular control systems. He proposed that genetic components were responsible for maintaining and perpetuating a library of genes, expressed and unexpressed, through a template replicating mechanism. He then deemed epigenetic components as auxiliary mechanisms that controlled the expression of specific genes (Nanney 1958a; Haig 2004, 2012). Most importantly, in addition to discussing variability in expression patterns, Nanney (1958a) emphasized the fact that expression states could persist through cell division. Although some have claimed that Nanney's usage of the term epigenetic was developed independently of Waddington's definition (he initially used the term *paragenetic*) (Haig 2004), considerable overlap can be found in their contemporary writings on genotype–phenotype relationships (Nanney *et al.* 1955, 1958a,b; Waddington 1939, 1942a,b), gene expression (Nanney *et al.* 1955, 1958a,b; Waddington 1939, 1942a.b), and the respective roles of the nucleus and the cytoplasm in gene regulation (Nanney 1953, 1957, 1958a; Waddington 1939, 1956). It is clear, however, that Nanney's contemplation of the stability of cellular expression states was an important addition to Waddington's ideas, which had significant impacts on the future direction of epigenetics. For a more detailed treatment of this history please refer to Haig (2004, 2012) and Holliday (1994).

Definitions of Epigenetics

It was largely through a shared interest in development and cellular differentiation that Waddington, Nanney, and others came to use the term *epigenetic*; however, the focus of those within the field did vary, with some, such as Waddington, being more concerned with gene regulation and genotype–phenotype interactions, and others, such as Nanney and Lederberg, being more interested in the stability of expression states and cellular inheritance. As stated by Haig (2004), interest in these different aspects of epigenetics led to a division within the field that can be directly linked to the definitional identity crisis that exists today.

Throughout the 1980s and 1990s, the definition of epigenetic moved farther away from developmental processes and became more generalized. For example, one definition from 1982 describes epigenetics as "pertaining to the interaction of genetic factors and the developmental processes through which the genotype is expressed in the phenotype" (Lincoln *et al.* 1982). This definition does include the term *developmental*, but its meaning seems to relate more to the development of the phenotype than to an ontological meaning. Although only slightly different from Waddington's original definition, this definition and others during this time broadened the meaning of epigenetics in important ways. It made the term more available and applicable to other fields by emphasizing the importance of genetic and nongenetic factors in controlling gene expression, while downplaying (although not ignoring) the connection to development (Medawar and Medawar 1983; Hall 1992; Jablonka and Lamb 2002).

Concurrently, research being done in the 1970s and 1980s on the relationship between DNA methylation, cellular differentiation, and gene expression (Holliday and Pugh 1975; Riggs 1975; Jones and Taylor 1980; Bird *et al.* 1985) became more closely associated with epigenetics. The work of Robin Holliday and others, on cellular memory and DNA methylation, particularly the finding that DNA methylation had strong

effects on gene expression and that these effects persisted through mitosis, corresponded to Nanney's (1958a,b) writings on the stability of expression states. This prompted Holliday to redefine epigenetics in a way that was more specific and squarely focused on the inheritance of expression states (while Nanney discussed epigenetic inheritance, his definition of epigenetics did not include a specific component on heritabililty). Holliday (1994) offered two definitions of epigenetics, both of which were admittedly insufficient when taken separately but comprehensive in covering all currently acknowledged epigenetic processes when taken together. The first definition posed that epigenetics was "the study of the changes in gene expression, which occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression." The second stated that epigenetics was "nuclear inheritance, which is not based on differences in DNA sequence." Wu and Morris (2001) streamlined Holliday's definition to state "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail change in DNA sequence."

The addition of heritability to Waddington's original definition by Holliday was a significant change. While Waddington's definition does not preclude the inheritance of expression states [indeed Waddington (1942a) did briefly discuss heritability in his paper "The Epigenotype"], this aspect was not a fundamental part of his concept of epigenetics. Despite the more thorough discussion of heritable expression states by Nanney and others, this was the first definition to make heritability a necessary part of epigenetics.

The implications of Holliday's redefinition were significant. The field soon became a residence for perplexing phenomena that didn't fit squarely into other genetic fields and, in many regards, the inability to explain these phenomena by simple genetic explanations became a defining element of epigenetics. Prior to understanding RNA-based regulatory mechanisms, and still in early stages of understanding DNA methylation and histone modifications, the decoupling of genotype and phenotype exemplified by epigenetics provided an attractive refuge because it offered metaphorical language to describe the disconnect between a gene and its phenotypic properties. This included occasions where the expression of a gene varied depending on its location (such as position effect variegation in *Drosophila* or yeast), history (imprinting), or other circumstances (*e.g.*, the establishment of centromeres, telomere healing prior to sequence addition). The thrill and charisma of a "new" genetics initiated a virtually unparalleled wave of interest in epigenetics over a very short amount of time (Cold Spring Harbor Symposium on Quantitative Biology 2004; Haig 2012).

The Problem

It is not difficult to find articles in the current scientific literature that use *epigenetic* to mean any one of the definitions above, or others entirely. It is futile to argue over the correctness of any one definition; however, it is important to acknowledge that the lack of a universal definition has produced significant ambiguity across biological fields. As previously acknowledged by Haig (2004) and others (Bird 2007; Haig 2012; Mann 2014), what we have today is a pronounced dichotomy within the field of epigenetics. Waddington's *epigenetics* describes the interplay of genetic and cytoplasmic elements that produce emergent phenotypes (Van Speybroeck 2002; Jamniczky *et al.* 2010), and those in the biological sciences interested in gene-by-environment interactions and phenotypic plasticity use the term in this sense. As a result, Waddington's definition is largely used to describe the expression of environmentally mediated phenotypes, particularly in the fields of ecology (Rollo 1994; Pigliucci 2007; Bossdorf *et al.* 2008) and physiology (Jablonka 2004; Aguilera *et al.* 2010; Ho and Burggren 2010). Those in the field of genetics concerned with DNA methylation, chromatin activity states, chromosomal imprinting, centromere function, etc., predominantly use Holliday's notion of epigenetics. They are interested in how expression patterns persist across different cells (mitosis) and generations (meiosis). The phenomena being described by these two groups, and more importantly the mechanisms underlying them, are vastly different, yet they both use the same term: *epigenetic*.

This ambiguity has made even the simple task of identifying epigenetic phenomena difficult and also constrains more advanced pursuits to determine how epigenetic processes occur. After all, how can scientists effectively study a process when they cannot even agree on how to define it? With the usage of the term *epigenetic* increasing exponentially across scientific and mainstream literature, one must wonder: for all the interest and attention epigenetics is receiving, why don't we have a clearer understanding of it?

The primary challenge is reconciling Waddington's epigenetics with Holliday's epigenetics, because while both exist, they may not necessarily be related to each other. Is there room within one field to entertain both definitions? Moreover, do the phenomena underlying each have any business being categorized together, particularly when their connection is based more on history and semantics than deliberation? Answering these questions is important for streamlining the field, facilitating more effective interchanges between researchers, and developing clearer research objectives.

The second challenge lies in addressing the methodological problems that have accumulated within the field of epigenetics over time, due to the absence of a clear definition. The principles that provide the foundation of any biological field exist to direct research and achieve objectives within that field; however, without this clear foundation, our desire to understand epigenetics has dictated our experimental approaches, colored our mechanistic interpretations, and allowed us to gloss over inadequacies. Rather than building from clear first principles, the field of epigenetics continues to be a catchall for puzzling genetic phenomena from which categorizations and justifications were developed *a posteriori*. Working backward to reevaluate

the first principles of epigenetics will help put the field on a stronger track and will hopefully allow research to flourish.

Ruminations on Important Terms: Dependence, DNA Sequence, and Heritability

Understanding why some genes are turned on or off is certainly less mysterious now than when the field of epigenetics was born, largely because of the identification of regulatory gene–gene and gene–protein interactions. These findings go a long way to explain the changes in gene expression that Waddington termed epigenetics, but the real difficulty is in satisfying Holliday's addendum of heritability. These regulatory components are all encoded by DNA; however, Holliday's conceptualization of epigenetics requires that the status of gene expression, not just the components needed for gene expression, be heritable. Also, this phenomenon requires an additional mode of inheritance that is not dependent on DNA sequence. To fully comprehend Holliday's definition, we must first make sure that all of the elements are accurately defined. This requires not only taking a critical look at how Holliday's description defines the terms *dependence*, *DNA sequence*, and *heritability*, but also the range of possible meanings.

Dependence

The term *dependence* carries several potential meanings. In a strict sense, any molecule that cannot exist in the absence of DNA could be considered to be dependent on DNA. Therefore, any molecule or process that relies on DNA for its creation, perpetuation, and/or activation is dependent, and this would include any molecule that requires DNA as a substrate. From this perspective, anything from DNA methyltransferases (DMNTs), which are expressed by specific *DMNT* genes, to histones, which use DNA as a substrate during modification, would be considered dependent on DNA.

It is likely, however, that Holliday and others would argue that this is not the meaning they had in mind when they made this distinction. Instead, they refer to dependence in a stricter sense as the relationship between the location of a *particular chromosomal locus*, *the specific base pair DNA sequence* within that locus, and a reliable *expression state* (Holliday 1994). For example, Holliday's argument is that the ability of the same DNA sequence to produce different expression profiles without a base pair change shows a lack of dependence on the primary sequence because something outside of the sequence must be controlling expression. This then requires that we understand what exactly is meant by *DNA sequence*.

DNA sequence

Many characteristics of DNA sequence are often overlooked and underappreciated. Most geneticists are primarily concerned with euchromatic regions containing sequences that make up genes and encode proteins. This isn't too surprising, given that these are the portions of DNA responsible for producing the majority of proteins vital to cell survival and function. Repetitive sequences, including those found in the heterochromatin, are often viewed as less important and commonly referred to as *junk DNA* (Ohno 1972; Brosius and Gould 1992; Kapranov and Laurent 2012; Graur *et al.* 2013). The ambivalence toward repetitive sequences likely stems from the fact that their function is poorly understood, and that the tools for investigating them are undeveloped. The bias toward protein-coding regions and the difficulty in working with repetitive sequences has shaped, and perhaps limited, our understanding of the role gene sequence plays in gene expression; however, there is evidence that other aspects of DNA, aside from the base pair sequence within gene regions, are important for gene expression.

One example is that the expression of a gene can be dependent on other sequences lying outside of the coding region (*cis*- and *trans*-regulatory elements or repetitive sequences). This makes it difficult to understand, and therefore reject, a relationship between gene expression and primary sequence because the expression of one gene may be dependent on the primary sequence of another section of DNA (see Figure 1). These problems are solved by expanding the definition of a gene to include regulatory elements and a rigorous requirement to map the genetic locus of regulatory changes. The former is easily accomplished (but often suffers from ambiguity and difficulties in precisely determining the boundaries of a gene), while the latter is rarely pursued in epigenetics literature.

A second, often overlooked characteristic of DNA sequence is location, which can impact gene expression in both coding and noncoding regions. Position-effect variegation (PEV) demonstrates that moving a gene sequence to a different location within the genome can affect its expression (Gowen and Gay 1934; Spofford 1976; Karpen 1994), and in these cases nondependence is still upheld by most epigeneticists as long as no changes occur in the transposed sequence. But why is the location of a gene sequence viewed as unimportant? To those who use transgenesis, a common practice in biology, it is abundantly clear that the location of an inserted transgene has significant effects on its expression (Al-Shawi *et al.* 1990; Wilson *et al.* 1990). In fact, Waddington explicitly promoted the idea of incorporating gene position and arrangement as an element of the genotype due to its important effects on expression (Waddington 1939).

A third salient characteristic of DNA sequence is the copy number of nearby sequences. Studies have shown that repeat regions can play important regulatory roles (Lemos *et al.* 2008; Zhou *et al.* 2012) and that the proximity of coding regions to repeats (Dorer and Henikoff 1997), as well as the size of the repeating regions (Howe *et al.* 1995; Paredes *et al.* 2011; Sentmanat and Elgin 2012), can have unique effects on gene expression and chromatin structure. This also means that changes in repeat regions,

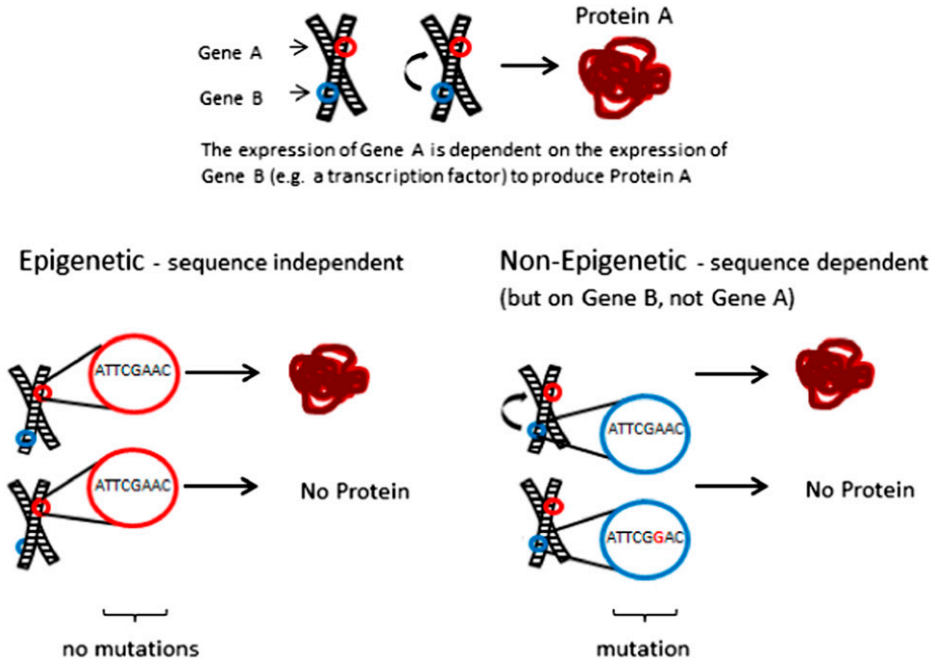

Figure 1 Imagine the expression of gene A is dependent on the expression of gene B (a transcription factor or si/piRNA perhaps). If we see variable expression in A, but no change in the sequence of gene A, we may conclude that this provides evidence for the expression of A being sequence independent and a product of epigenetics, as shown below. However, it is possible that sequence changes have occurred in gene B, producing transcriptional changes in A. This would make the expression of A *dependent* on the primary sequence of gene B but not the sequence of A itself. This makes the task of proving sequence independence difficult because you cannot simply look for sequence changes in the coding region of the gene in question, but must also be sure expressional changes aren't due to mutations elsewhere on the chromosome or other places in the genome.

which are notoriously difficult to detect, must also be ruled out to accurately show sequence independence.

Heritability

Perhaps the most important and definitive element found among definitions of epigenetics, is the *heritability* of expression states. With this addition one could argue that the definition of epigenetics was simultaneously expanded and constricted. On the one hand, incorporating heritability into the discussion forces us to consider epigenetics on a more conceptual level by thinking about the role of time and the relationship between the stimulus that causes an expressional change and the lasting or fleeting effects of that change. On the other hand, requiring that expressional changes persist through mitosis and/or meiosis in order for a phenotype to be considered epigenetic drastically reduces the number of observations that qualify. For these reasons, this aspect of Holliday's definition is the most controversial, particularly since it requires the acknowledgment of a new mode of inheritance.

From a semantics perspective, the inclusion of heritability also expands the meaning of the term itself, which has traditionally related to the transfer of only DNA. Using heritability to describe the transfer of non-DNA molecules, whether they are methyl groups, histones, or cytoplasmic compounds, broadens the concept of inheritance in an intriguing way. However, Holliday's definition doesn't actually delineate the difference between the inheritance of molecules and the transfer of molecules, nor does it state what kind of molecules can and cannot be inherited. Without this distinction it is very difficult to separate epigenetic phenomena from nonepigenetic phenomena, and also to investigate how such modes of inheritance may function.

Holliday's concept of *heritability* also produces several complications in practice. First, it can be surprisingly difficult to discern between changes in gene expression due to the inheritance of an expression state and those due to a real-time reaction to a stimulus. To show that an expression state is inherited, you first need to have a clear understanding of the cause (*i.e.*, stimulus). Knowing the relationship between a given stimulus and its expressional effect(s) is paramount to creating a timeline and conclusively showing that a barrier exists between the two for which inheritance in necessary. For example, this would entail that a parent cell or organism experienced a stimulus that caused a specific expression pattern and then that a similar expression pattern was also evident in the offspring without the offspring having ever experienced the initial stimulus.

While these connections are easy enough to conceptualize, they can be difficult to prove empirically, not only because gene expression can be capricious, but because in many cases the stimuli impacting a parent also may impact the germ cells residing in the parent, germ cells which will ultimately go on to produce daughter cells and/or offspring. If the germ cells respond to a stimulus experienced by the parent, no barrier exists between the stimulus and offspring because expression in the primordial cells of the future offspring are also directly affected. For example, in mammals, any stimuli impacting a pregnant female carrying daughters may impact the mother, the fetus, and the germ cells of the fetus, which will go on to produce offspring (Youngson and Whitelaw 2008; Daxinger and Whitelaw 2012; Dias and Ressler 2014). This means that any stimulus experienced by the mother may also result in direct exposure to two additional generations of potential offspring. In this scenario, one would have to show a similarity in expression between the mother and her great granddaughter to verify a possible epigenetic connection (Skinner 2007; Skinner *et al.* 2013). However, if the expression pattern of the original germ cell were apparent in the offspring, it would still

satisfy Holliday's definition, as persistence through mitosis would have had to occur (Holliday 1994). This has led to some clarifications in the identification of epigenetic phenomena, but those attempts have yet to clearly delineate Waddington's and Holliday's views (Youngson and Whitelaw 2008; Berger *et al.* 2009; Grossniklaus *et al.* 2013; Dias and Ressler 2014).

The primary difficulty lies in identifying the mechanism of inheritance. Do the compounds responsible for perpetuating an expression pattern have to be closely associated with DNA, as in methylation and chromatin modification, or do cytoplasmic compounds qualify? If so, should the transfer of cytoplasmic compounds really be considered inheritance? Waddington stressed the importance of cytoplasmic compounds and their effect on gene expression (Waddington 1935), yet maternal or transgenerational effects mediated by cytoplasmic transfer from mother to offspring would not be considered epigenetic under Holliday's definition because the expression pattern of the offspring is not independent and simply results from the transfer of cytoplasmic compounds, such as RNA, transcription factors, prions, etc. (Ptashne 2008; Jarosz *et al.* 2014). These issues make the contrast between Waddington's epigenetics and Holliday's epigenetics much more evident.

Possible Solutions

The ambiguity surrounding the field of epigenetics, as well as the historical basis for this definitional confusion, has been discussed by many over the last 15 years (Holliday 2002, 2006; Jablonka and Lamb 2002; Haig 2004; Bird 2007; Berger *et al.* 2009; Mann 2014). This has led to the development of several new definitions and terms to help clarify the issue. Bird (2007) proposed that epigenetics could be redefined as "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states," a definition that he feels unified Holliday's requirement for heritability with Waddington's more general definition. Mann (2014) also advocated keeping a broad notion of epigenetics, but offered the term "memigenetic" to denote expression states that are heritable. Despite these suggestions, a strong working definition for epigenetics has yet to be adopted, and we believe that this largely results from (1) attempting to combine Waddington's and Holliday's definitions into one comprehensive term and (2) the absence of specific terms within the available definitions that identify the mechanistic components underlying epigenetic phenomenon.

We don't feel that it is possible to reconcile Waddington's focus on gene regulation with Holliday's more specific criteria within one field and still maintain the level of clarity needed to produce a useful definition. The efforts to preserve a relationship between these two conceptualizations have been impaired by the fact that there are just too many phenomena, with too few mechanistic connections, to categorize into one field. Also, among the definitions that do maintain the requirement of heritability, we feel that many lack the detail to be functionally useful in directing the testing of specific hypotheses, particularly as it relates to the location or site (cytoplasm or nucleus) of epigenetic phenomena. To mitigate these shortcomings, we advocate defining epigenetics as "the study of phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression that are not dependent on changes to DNA sequence."

We feel that this definition makes a strong distinction between gene regulation (Waddington's definition) and epigenetic inheritance (Holliday's definition), and also emphasizes that epigenetic phenomena must deal exclusively with chromosome-bound changes. By making these distinctions, we have efficiently separated expressional changes caused by cytoplasmic compounds, which are more closely tied to gene regulation, from those which occur on, or in close association to, the chromosome. Doing so makes the focus of the field much clearer and identifies epigenetic mechanisms more explicitly.

We feel that this definition touches on several important elements not encompassed by other definitions, yet commonly implied in most uses. To further explain the reasoning behind our definition, as well as its utility for improving epigenetic research, we would like to offer a *clarification* and a *test*.

The Clarification

In the battle between Waddington and Holliday's definitions, we have clearly chosen Holliday's conceptualization, and this has occurred for two reasons. First, although the usage of Waddington's general definition has increased within nongenetic fields, particularly ecology and physiology, to describe environmentally mediated phenotypes and trait plasticity, we feel that these topics fall more clearly under the heading of gene regulation. Second, the phenomena that pose the most serious challenges to traditional genetic theory, which dictates that identical sequences should behave identically, are genomic imprinting, X inactivation in mammals, centromere/telomere establishment and stability (McClintock 1939; Ahmad and Golic 1998; Barry *et al.* 2000; Maggert and Karpen 2001; Blasco 2007; Black and Cleveland 2011; Mendiburo *et al.* 2011), and perhaps others. Most of the work on these issues has and continues to occur in the field of genetics, and we believe that the epigenetics fits most appropriately within the realm of genetics, given this strong precedent of research. That being said, we do want to clarify some points regarding Holliday's definition and the current state of the field of epigenetics.

Holliday's addendum on heritable expression states arose as a *hypothesis* to explain the phenomena listed above; however, rather than this hypothesis being thoroughly tested, it quickly perpetuated several new ideas regarding potential mechanisms for inheritance (methylation, histone modifications, *etc*.) without strong empirical proof for the necessity of such mechanisms. Although Holliday's ideas on the perpetuation of expression states and cell memory are innovative and may very well prove to be accurate, we feel an important step in the process of developing these ideas has been overlooked. This is particularly true when the attempts to validate

these hypotheses have, as of yet, proved inconclusive. What can it mean to say that DNA methylation is repressive when activation of a gene removes methylation (*e.g.*, Bird 2002; Nagae *et al.* 2011; Hackett *et al.* 2012; Qian *et al.* 2012; Gan *et al.* 2013; Xie *et al.* 2013; Bestor *et al.* 2014)? The search for the mechanism of semiconservative histone modifications continues (Deal *et al.* 2010; Xu *et al.* 2010; Nakano *et al.* 2011; Tran *et al.* 2012; Whitehouse and Smith 2013) despite evidence that the modifications respond to expression state rather than control it (Kilpinen *et al.* 2013; Ptashne 2014; Teves *et al.* 2014). It's not that histone modification and DNA methylation are not correlated with gene expression differences—they are—but the possibility that they may be responsive rather than causal has not been disproved (Henikoff 2005; Ptashne 2013). We include causation in our definition to reflect these shortcomings, in acknowledgment of the inadequacies in sequencing repeat regions and the conceptualization of important terms (*DNA sequence* and *heritability*) discussed earlier, and as an attempt to spur research that focuses on these fundamental issues.

The definition of epigenetics proposed above contains the necessarily vague "gene expression" so as to not exclude *a priori* any units of inheritance, including protein-encioding genes, telomeres, centromeres, functional RNA gene products (such as the rRNA, miRNAs, pi/siRNAs, etc), origins of replication, G-quartets, genome instabilities, or anything else that can manifest a phenotype. Our explicit addition of "chromosome bound" encompasses the already- implied popular use of the term epigenetic, where local changes in gene expression are induced and inherited *at the specific gene being regulated*. This explicit statement added to Holliday's (1994) definitions, later merged by Wu and Morris (2001), assures two things. First, that epigenetics is not inferred from cytoplasmic or nucleoplasmic factors, *e.g.* perdurance of a proteinaceous transcription factor (Ptashne 2013). Second, that *heritable memory* (rather than "inheritance") is an explicit property of epigenetic gene regulation. The most heavily cited examples of epigenetic phenomena (*e.g.*, genomic imprinting) fulfill these criteria, and other cases that are more dubious (*e.g.*, stress-sensitivity in offspring of stressed pregnant mammal mothers) are excluded until better understood.

The Test(s)

To make the strong claim of sequence independence, one must assure that there are no changes to any sequence in *cis* or in *trans* to the gene whose expression is being monitored. Ideally, one would sequence the entire genome, yet this is impractical on many grounds, not least of which are the large blocks of repetitive heterochromatin on most chromosomes, which modern molecular biology cannot assemble (and thus modern molecular biologists tend to ignore). Instead, careful (and laborious) work, such as that done by some (Brink 1956; Clark and Carbon 1985; Steiner and Clarke 1994; De Vanssay *et al.* 2012) showing frequent switching, should be considered strong evidence in the place of exhaustive sequencing. We must, however, always be concerned with the possibility of efficient inducible changes masquerading as "epigenetic" cases, *e.g.*, mating type switching in yeasts (Haber 1998), VDJ recombination (Blackwell and Alt 1989), repeat-sequence instability (Hawley and Marcus 1989), and induced mutation (McClintock 1983; Piacentini *et al.* 2014); after all, they do bear all of the hallmarks of epigenetic changes save one: we happen to know their mechanism. For that reason, it is critical to refrain from negative claims (that is, assertions of "no difference") as implied in "genetically identical chromosomes," when chromosomes have not been sequenced. Ideally, one should be able to make strong positive statements to conclude epigenetic gene regulation is at play.

One can experimentally test for sequence independence using a genetic approach. If we regard an expression state as a phenotype (and indeed Holliday's, and Wu and Morris's definitions clearly make mRNA production a phenotype), then it is a simple matter to map a phenotype to the location on the chromosome it stems from. In the example of A and B in Figure 1, if the stable expression state of A maps to the physical location of A on the chromosome, then we can have confidence that the expression state is a consequence of some feature (perhaps epigenetic) of A. Subsequent work showing lack of sequence dependency would confirm epigenetic regulation. If however, the status of A maps to the B locus, or to the heterochromatin, or even to the nucleoplasm, then there is no reason (and in fact no justification) to claim that A's expression state is epigenetic. It is likely instead controlled, through well-understood mechanisms, *e.g.*, by the presence of another factor (Ptashne 2013; Serra *et al.* 2014; Struhl 2014). In these cases, there is nothing meaningfully "dependent" about the "sequence" of A in terms of its regulation.

At an ideal extreme, identical reporter sequences should be placed in the same nucleus (through transgenesis or mating). If a regulatory change is epigenetic, then those sequences should (or could) behave differently, each independently maintaining a memory of their states. This idea is the intellectual foundation of the search for heritable histone modifications, DNA methylation, etc., yet is rarely directly tested. Strikingly, and underscoring our concern, in a few cases where data have been presented, the idea of allele-specific memory is either not tested or is directly refuted (Anway *et al.* 2005; Pembrey *et al.* 2006; Greer *et al.* 2011; Crews *et al.* 2012; Stern *et al.* 2012; Voutounou *et al.* 2012; Buescher *et al.* 2013; Padmanabhan *et al.* 2013; Wan *et al.* 2013; Gapp *et al.* 2014).

These conditions—nonsimilar behavior of identical sequences, mapping of the epigenetic state—are implied by most uses of the term epigenetic. Importantly, they are taken to imply a great deal about how gene expression works, suggesting that there is an entire layer of gene regulation that we are only now becoming aware of. Or is there? Before we rewrite the textbooks, divert funding initiatives, refocus our disease intervention strategies, or alter our view of neo-Darwinian biology, it is our obligation to attempt these simple tests to assure ourselves that we are not chasing a ghost.

Conclusions

The legacy of Waddington, and later Holliday and others, has enriched our understanding of chromatin structure, gene expression, and the environmental influence and nondeterministic capabilities of genes. However, without understanding the history of the term epigenetic, and the baggage that comes along with its different uses, we run real risks in biology. While gene expression, DNA methylation, regulatory RNAs, histone modifications, mitotic stability, and transgenerational inheritance are all correlated and intertwined, we must absolutely resist the temptation to equate them all mechanistically. We must utterly reject the notion that what we learn in one case (the mitotic inheritance of DNA methylation patterns at genomically imprinted control regions) are predictive of the properties of other cases (methylation causes inducible and meiotically heritable changes to mRNA transcription states) simply because they share the same ill-defined term, "epigenetics."

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PERSPECTIVE

An operational definition of epigenetics

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A recent meeting (December 2008) regarding chromatin-based epigenetics was hosted by the Banbury Conference Center and Cold Spring Harbor Laboratory. The intent was to discuss aspects of epigenetic control of genomic function, and to arrive at a consensus definition of "epigenetics" to be considered by the broader community. It was evident that multiple mechanistic steps lead to the stable heritance of the epigenetic phenotype. Below we provide our view and interpretation of the proceedings at the meeting.

Definition: "An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence."

The definition of epigenetics proposed here, as with the classical definition (e.g., as proposed by Conrad Waddington in the 1950s), can involve the heritability of a phenotype, passed on through either mitosis or meiosis. Understanding the mechanisms involved in the initiation, maintenance, and heritability of epigenetic states is an important aspect of research in current biology. Several distinct but interconnected molecular pathways have been discovered to date. Below is described a set of operational steps in which such pathways can be placed, in an effort to define the different mechanistic aspects of epigenetic transmission.

Epigenators, Initiators, and Maintainers of the epigenetic process

It is proposed here that there are three categories of signals that culminate in the establishment of a stably heritable epigenetic state: a signal that we propose to call the "Epigenator," which emanates from the environment and triggers an intracellular pathway; an "Epigenetic Initiator" signal, which responds to the Epigenator and is necessary to define the precise location of the epigenetic chromatin environment; and an "Epigenetic Maintainer" signal, which sustains the chromatin environment in the first and subsequent generations. These classes are depicted in Figure 1 and are explained below.

Epigenator

The epigenetic phenotype is likely triggered by changes in the environment of the cell. Everything occurring upstream of the first event on the chromosome would be part of the Epigenator signal, including an environmental cue or niche and the subsequent signaling pathways leading to the Initiator. Once an Epigenator signal is received, it is converted to an intracellular Epigenator pathway culminating in the "activation" of the Initiator. The Epigenator signaling pathway could be a protein–protein interaction or a modification-based event that unleashes the latent activity of the Initiator. The Epigenator signal will be transient, remaining in the cell long enough to trigger the epigenetic phenotype but not necessary for subsequent events.

Epigenetic Initiator

The Initiator translates the Epigenator signal to mediate the establishment of a local chromatin context at a precise location. Following the priming of the Initiator by the Epigenator signal, the Initiator will define the location on a chromosome where the epigenetic chromatin state is to be established. The Initiator could be a DNA-binding protein, a noncoding RNA, or any other entity that can define the coordinates of the chromatin structure to be assembled. Consequently, some form of sequence recognition must be a feature of this signal. The Initiator will in general be a signal that requires self-reinforcement and self-renewal through positive feedback mechanisms. One operational characteristic of the Initiator is that it may be sufficient to initiate an epigenetic phenotype when introduced into a cell. Also, unlike the Epigenator, the Initiator may not dissipate after its action, but rather may persist with the Maintainer.

Epigenetic Maintainer

The Maintainer sustains the epigenetic chromatin state but is not sufficient to initiate it. This signal involves many different pathways, including DNA methylation, histone modifications, histone variants, nucleosome positioning, and others. Maintainers have the common

[*Keywords*: Epigenetics; DNA methylation; noncoding RNA; histone modification; histone variant]

[5]**These authors contributed equally to this work.**

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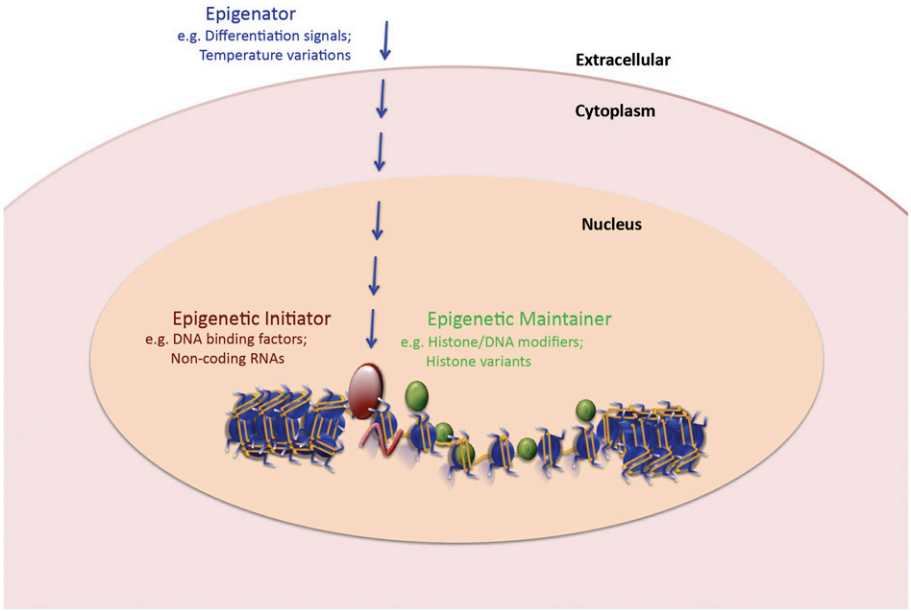

Figure 1. The epigenetic pathway. Three categories of signals are proposed to operate in the establishment of a stably heritable epigenetic state. An extracellular signal referred to as the "Epigenator" (shown in blue) originates from the environment and can trigger the start of the epigenetic pathway. The "Epigenetic Initiator" (shown in red) receives the signal from the "Epigenator" and is capable of determining the precise chromatin location and/or DNA environment for the establishment of the epigenetic pathway. The "Epigenetic Maintainer" (shown in green) functions to sustain the chromatin environment in the initial and succeeding generations. Persistence of the chromatin milieu may require cooperation between the Initiator and the Maintainer. Examples for each category are shown *below* each heading. Chromatin is depicted in blue.

property that they do not have absolute DNA sequence specificity. Consequently, they could operate at any chromosomal location to which they are recruited by an Initiator. Maintainers may function by carrying an epigenetic signal through the cell cycle or could maintain epigenetic landscapes in terminally differentiated cell types.

The role of one particular class of potential Maintenance signals—i.e., post-translational modifications of histone proteins—requires particular clarification. During the meeting, several examples for an epigenetic role of histone modifications were presented. These included roles of (1) H3K4 and H3K27 methylation, by trithorax and polycomb complexes, respectively, in homeotic gene expression; (2) H3K9 and H4K20 methylation in establishing memory of transcriptional silencing; and (3) H4K16 acetylation in mating-type behavior and aging in *Saccharomyces cerevisiae*. However, the term "epigenetic" is not always a correct term to define histone modifications. Many modifications play a role in more dynamic processes such as transcriptional induction and DNA repair. Thus, certain histone modifications very likely play a role as Maintainers of epigenetic signals; however, this does not mean that all post-translational modifications of histones are epigenetic in nature.

Biological examples

There are not many well-defined examples of Epigenators. The best example comes from plants, where environmental signals such as temperature affect the epigenetic process of paramutation. Examples of Initiators are noncoding Xist RNA, which is sufficient for silencing the mammalian X chromosome, and DNA-binding factors that lead to reprogramming of differentiated cells into stem cells in metazoans. Maintainers include histones deacetylated by the Sir complex that functions in mating-type switching and sexual differentiation in yeast *S. cerevisiae*, DNA methylation at CpG islands in plants and some animals, and the histone variant CENPA at centromeres of all eukaryotes.

Final remarks

Epigenetic events in eukaryotic organisms have evolved to provide a more precise and stable control of gene expression and genomic regulation through multiple generations. This is exemplified by the existence of sex-specific dosage compensation or the fine-tuning of allele-specific expression, as seen in imprinted loci. Deregulation of such processes may lead to disease; e.g., misregulation of imprinted genes results in the genesis of Beckwith-Wiedemann and Prader-Willi/Angelman syndromes, whereas the loss of other epigenetic heritance mechanisms results in cellular aging and cancer. In addition, the ability to epigenetically reprogram differentiated cells is becoming of medical importance.

The effort by the meeting participants to define and discuss "epigenetics" was an attempt to add focus and clarity to this exciting and growing area of research.

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