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RESEARCH ARTICLE

Chromatin research and epigenetics - historical perspectives, current research, open questions, and misconceptions

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Abstract

The concept of chromatin as a complex of nucleic acid and proteins in the cell nucleus was developed by cytologists and biochemists in the late 19th century. It was the starting point for biochemical research on DNA and nuclear proteins. Interest in chromatin declined rapidly at the beginning of the 20th century, but a few decades later a new focus on chromatin emerged, which was not only related to its structure, but also to its function in gene regulatory processes in the development of higher organisms. Since the late 20th century, research on chromatin modifications as well as DNA methylation that emerged in the 1970s have also been conducted under the label epigenetics, a term originally introduced for the complex processes between genotype and phenotype during development. These processes - in particular gene regulation - were subsequently scrutinized by molecular biologists.

Research termed epigenetics remained marginal until the end of the 20th century but experienced a rapid rise when heritability was added to its definition. This was accompanied by an increasing diversity in researchers' understanding and definitions of epigenetics. Epigenetics now includes research on histone and DNA-modifying enzymes, nucleosome remodelers, histone chaperones, chromatin-binding proteins to facilitate transcription factor and polymerase action, and the role of long non-coding RNA and small interfering RNA in transcriptional regulation.

This article highlights the major phases of chromatin and epigenetics research until the present time and illuminates how different scientific contexts changed the relevance and meaning of chromatin from the 19th century. The paper also points to misconceptions and media hype about epigenetics, for example unsupported claims about transgenerational inheritance in humans or questioning of the basic biological principles of gene regulation based on specific regulatory sequences of the genome.

Keywords: chromatin structure, diversity of epigenetics, gene regulation, transcription factors, epigenetics media hype

Introduction

The correct expression of genes in the cell or in tissues at the right time is fundamental for the development and functioning of an organism. Geneticists proposed a rough idea of differential gene expression already in the 1930s. Interestingly, ideas of how structural features of gene environments might affect gene activities were also discussed at the time.

Chromatin, now understood as a nucleoprotein complex into which eukaryotic genomes are packaged, was first described by cytologists ca. 150 years ago, when dyes were available from the chemical industry, and the microscopy and biochemistry of the cell had made major progress. Biochemists shortly after defined chromatin as an association of DNA and alkaline proteins, a definition that has remained valid until today. Chromatin is now known to play an important role in the highly complex machinery of gene regulation in eukaryotes that includes several protein complexes and long non-coding RNA.

The term epigenetics that was introduced in 1942 for the causal interactions between genes and their products during development, has drastically changed its meaning over time. It is now used for a wide range of research related to the complex machinery of gene regulation that includes chromatin modifications by methyl-, acetyl and other groups. Research labelled epigenetics rose strongly only in the 21st century.

This article reviews major steps in chromatin research and epigenetics, points to the role of chromatin in the complex processes of gene regulation, and critically analyzes some of the widespread unsupported claims by some epigeneticists and the public that aim at relativizing the central role of DNA sequences in these processes. I conclude by critically evaluating assertions of epigenetics constituting a revolution in biology.

1. Chromatin research – from cytology to epigenetics

1.1 Cytology and genetics

In 1869, physiological chemist Friedrich Miescher isolated *nuclein* from the nuclei of lymphocytes. *Nuclein* was a phosphorus-containing high molecular substance consisting of an organic acid, which was called *nucleic acid,* later DNA, and alkaline proteins, later called histone proteins. At the same time, the improvement in microscopes, together with new dyes available from the dye industry, provided powerful tools to study the morphology of the behavior of chromosomes in cell divisions. In 1879, the term chromatin was introduced by cytologist Walther Flemming to describe the easily stainable threads in the nucleus while observing the processes of mitosis in a light microscope. Flemming suggested that chromatin (derived from the Greek word *chroma* (colour) might be identical with *nuclein,* an assumption that turned out to be correct. The chemical nature of chromatin or *nuclein,* i.e., a nucleic acid, which was later called DNA, and proteins, was already elucidated at the end of the 19th century. The term *chromosome* was used for the rod-like chromatin segments visible during mitosis.

In 1888, cytologist and embryologist Theodor Boveri proposed the theories of the continuity of chromosomes over the cell cycle (despite their becoming invisible between cell divisions) and their individuality. These theories became the basis for the recognition of chromosomes as causal factors of heredity and development. The first to understand the far-reaching implications of Boveri's theories was cytologist Edmund B. Wilson, who, by the end of the 19th century, attributed to *nuclein* or chromatin a central function in heredity, as is expressed in his often-cited statement: "There is, therefore, considerable ground for the hypothesis that in a chemical sense this substance [*nuclein* or chromatin] is the most essential nuclear element handed on from cell to cell, whether by cell division or fertilization".[1,2] Wilson also understood that chromosomes possessed an internal organization and varied not only from species to species but also from one chromosome to another in the same species.

The rise of Mendelian genetics at the beginning of the 20th century shifted the focus of interest away from the morphological and physical basis of heredity to the study of the effects of single genes and their location on chromosomes. Genes became abstract units, and the question of their material nature became marginalized.

From the 1920s, some geneticists studied genes also as part of their chromosomal environment, among them Alfred Sturtevant and Hermann Muller. In 1925 Sturtevant was the first to describe what came to be called "position effect," or the dependence of the functioning of a gene upon where in the chromosome it is located. During his studies of Drosophila chromosomes that underwent partial rearrangement as a result of irradiation, Muller in the 1930s generalized this phenomenon. He offered the far-reaching interpretation that the "functioning of the gene is affected by its shape and that this, in turn, varies with the strength and nature of synaptic forces acting on the region of the chromosome in which it lies."[3] Synaptic forces meant a physical connection due to forces exerted on the gene by other genes, or as a result of the spiralization of the chromosome region in question. The longitudinal differentiation of chromosomes in two types of chromatin that can be distinguished by their degree of condensation and stainability by dyes was established between 1928 and 1935 by cytogeneticist Emil Heitz: The less stainable and less condensed euchromatin was considered genetically more active, and the more stainable and condensed heterochromatin was believed to be genetically inert.[4] Assumptions as to the dependence of gene activity on genes' vicinity to heterochromatin were examined, but the question of whether a gene's location impacts its activity has not yet been conclusively answered. According to Jost et al.[5], the position of the gene inside or outside of heterochromatin and gene expression are not convincingly linked. The authors also make it clear that the cytological and molecular definitions of (hetero) chromatin have not been convincingly linked together.

1.2 Biochemistry

In contrast to genetic and cytological studies, biochemical research on chromatin has stagnated since the early 20th century. The exact chemical composition of DNA and the structure of a tetranucleotide were elucidated by the end of the 1920s, but the rapid development and success of classical genetics, which was based on genes as abstract entities, as well as the rise of colloidal biochemistry with its focus on unspecific molecular aggregates, had marginalized research on DNA and the proteins of chromatin.[6,7] The new interest in the chemical basis of heredity in the 1930s that led to the flourishing of molecular genetics, was based on research in bacteria and viruses that do not contain chromatin, because their chromosomes or DNA, respectively, do not include complexes with proteins. Only when, in the 1960s, molecular biologists began to explore more complex systems, such as eukaryotic cells and development, was research into the structural analysis and function of chromatin resumed with a focus on histone modifications.

1.2.1 Histone modifications

The pioneers of modern chromatin research were Vincent Allfrey and Alfred Mirsky, who in the 1960s confirmed histones' inhibitory effect on transcription and showed that their modifications by acetylation and methylation apparently alleviated this effect.[8] However, they could not show whether this was a causal relationship or an accidental correlation. According to Eric Davidson, a former PhD student of Mirsky's, histones were general, nonspecific inhibitors of genes, a notion that has become generally accepted; he suggested that gene regulation in eukaryotes would mainly come about by gene-controlled specific activators.[9] The discovery and chemical characterization of nucleosomes by R. Kornberg and J. Thomas and their microscopic identification by D.E. Olins and A.L. Olins [8,10,11] led to a strong rise in structural chromatin research in the early 1970s.[12] However, expectations at the time that their bead-on-a-string structure had a functional meaning did not come true.

Nucleosomes consist of DNA, wrapped around a protein core of histones, and are the smallest scale organizational unit of eukaryotic chromatin (figure 1). Their discovery played an important role in identifying the histones of chromatin. The core of the nucleosome contains an octamer of four different histones. Two super-helical turns of DNA are wrapped around the core and locked in place by the fifth histone. These "bead-on-a-string structure folds up into several higher orders of compaction".[10]

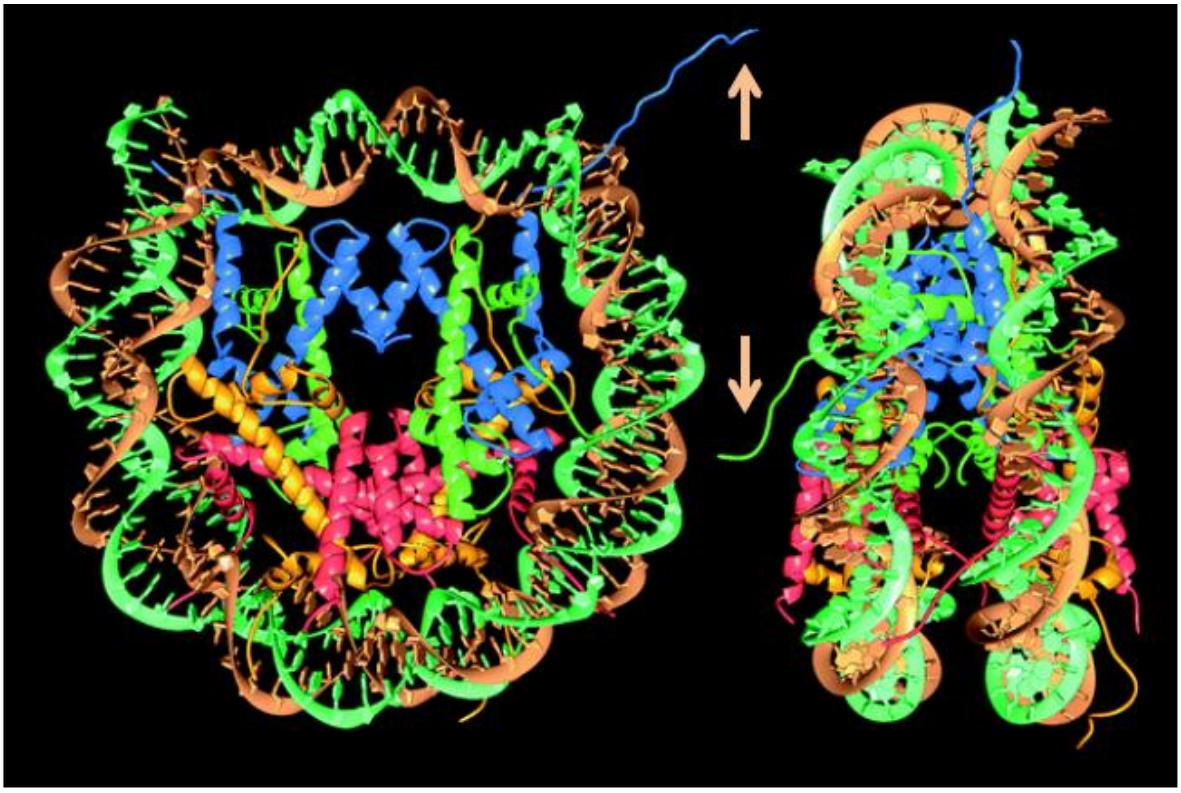

Figure 1

High-resolution structure of the nucleosome core particle. Individual histones are in blue, green, yellow and red. The arrows point to histone "tails" which are accessible for modification.
Source: Reprinted from Felsenfeld 2014, with kind permission of the author.

These higher orders of structure are considered to be a means to compact the DNA that was not needed for function in certain cells and therefore did not have to be opened for transcription, though this question has not been finally settled. It is also known that chromatin organization stabilizes and protects DNA, for example in mechanical processes such as mitosis. But, as was emphasized by Gary Felsenfeld, it soon became clear that chromatin also plays a major role at the level of the nucleosomes themselves. New questions were asked, such as, whether the histones had to be removed, or chromatin structure had to be modified, to enable transcription, and what roles chromatin played in the control of gene expression and DNA replication. This new research on chromatin structure modification started in the 1990s. The connection between chromatin structure and its function was first established in yeast, where a mutation of enzymes responsible for histone acetylation had a direct effect on yeast growth. Enzymes that move the nucleosomes around on DNA, chromatin remodeling enzymes, which had significant phenotypes, were also detected through yeast genetics.[10]

Histone modifications consist of small molecules, mostly methyl or acetyl groups, that are bound to certain residues of histone

tails. The responsible enzymes are not DNA sequence specific, i.e., they need DNA specific factors to recognize the histones in question. Histone modifications are sometimes transmitted by cell division, and in rare cases also by the germ line. However, the modifications are not stable and not faithfully copied, and they disappear after a few cell generations. As of now, there is no evidence that these modifications affect gene activity, but there is ample evidence that histone acetylation is generated as a consequence of transcription: The binding of transcription factors precedes histone acetylation that then reduces the affinity of the histone octamer for DNA, thus rendering transcription events easier (figure 2).[10]

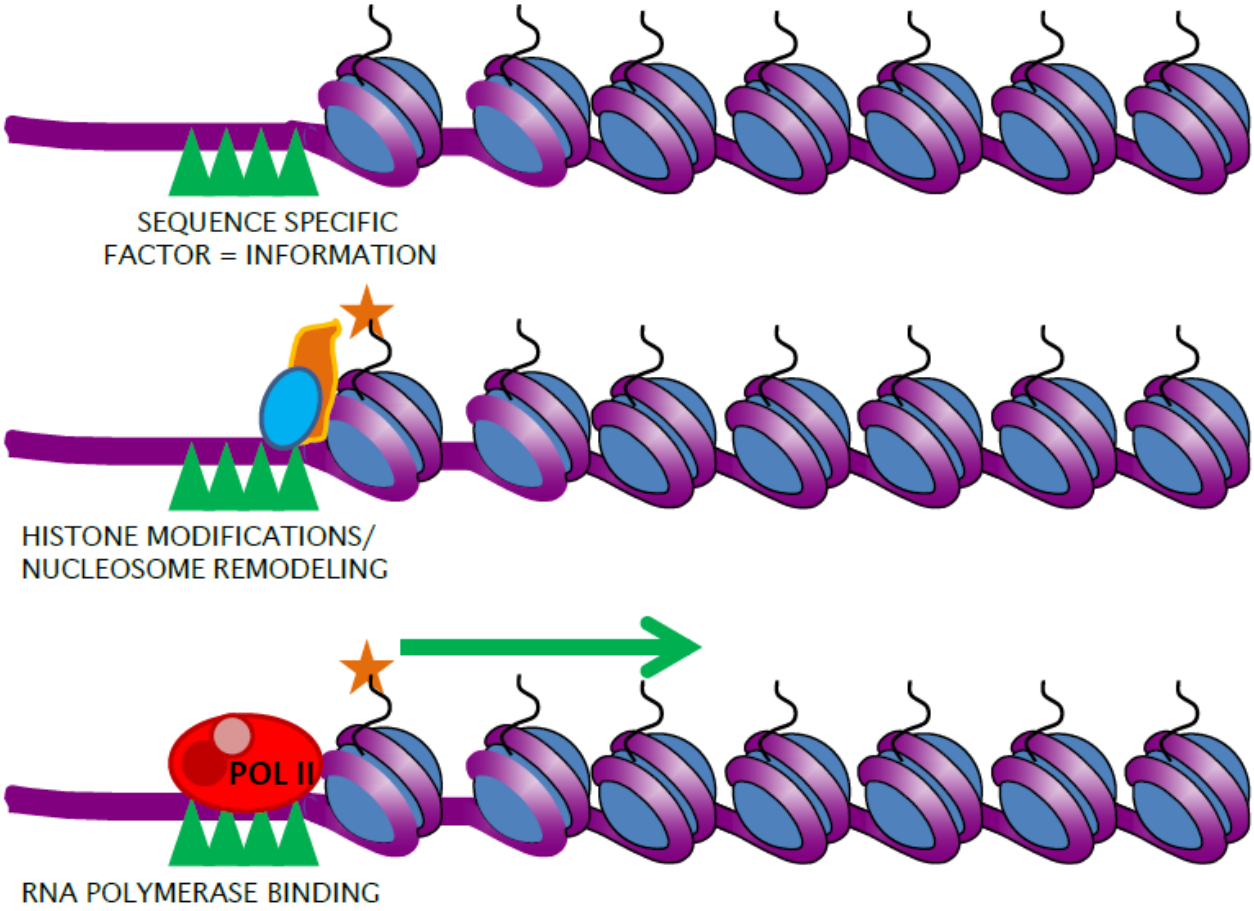

Figure 2

A simplified description of steps that may be associated with activation of gene transcription. The initial step (*top*) must involve recognition of DNA sites (nucleotide sequences) near the gene by one or more specific regulatory proteins that target that gene. That is the signal that allows the remaining steps (*below*) to prepare the chromatin to accommodate RNA polymerase and transcription.

Source: Reprinted from Felsenfeld 2014, with kind permission of the author.

These developments show that the structure-function relationship of chromatin is complex and not clearly understood. Unlike the case with DNA, where the elucidation of the chemical composition and structure was instrumental for understanding its function - identical replication, mutation, and information storage - the discovery of the nucleosome structure did not generate ideas about chromatin function that were not already known. Possible biological functions of chromatin in gene regulation were demonstrated by subsequent genetic research. The solution of the structure of individual histones turned out to be of no interest, because in vivo, histones only exist as part of a complex with other histones.[10] The idea of a histone code has met with skepticism, and the notion that the location of a gene on the chromosome is of greater importance than that of the surrounding regulatory DNA sequences was disproved unanimously. Despite these uncertainties, so Felsenfeld, it was clearly shown that histone modifications play an important role in transcription mechanisms, and interference with the histone modification process has multiple effects on the phenotype.

1.2.2 DNA and histone methylation

Since DNA and histone methylation acts through changing the chromatin structure, it is another example of modern chromatin research. In the late 1970s, Howard Cedar and Aharon Razin showed in *in vitro* experiments that DNA methylation caused gene repression.[13,14] Cedar emphasized that the changes in DNA methylation during development that are taking place from the time of implantation are specific and have to be directed by transcription factors or repressor proteins. An example is the complicated process of the turning off of pluripotency genes during cell type differentiation. It begins with DNA sequence-specific protein repressors at a time when these genes are still unmethylated and the chromatin structure is open. In the second step, chromatin becomes closed by particular proteins and enzymes are recruited to this site by the repressor. In the third step, the same chromatin system also recruits *de novo* DNA methylase. Only then do these genes undergo DNA methylation.[13] Cedar made it clear that methylation itself does not turn off a gene but contributes to keeping it silent, and demethylation itself does not turn on a gene but contributes to maintaining it active (see below). As soon as the transcription factors touch down on the gene, they are capable of bringing the machinery to open the chromatin and perform demethylation. In the 1980s, Timothy Bestor and his collaborators purified, characterized, and cloned the first eukaryotic DNA methyltransferase. By disrupting the gene that encodes this enzyme, they showed that DNA methylation is necessary for the suppression of transposons, while Rudolf Jaenisch, with whom Bestor collaborated, showed that DNA methylation is required for imprinted gene expression and X chromosome inactivation.

The role of DNA methylation and histone modification in the biochemical events that regulate genes is still not clearly established and is controversial. There are groups of

organisms such as nematodes and certain insects, such as Drosophila, which do not methylate their genomes. It is undisputed that DNA methylation does not silence active promoters of genes (DNA regions where transcription is initiated) but affects genes that are already silent.[15,13] It is also known that eukaryotic cells use many mechanisms to shut down and maintain repression of gene activity. Serena Sanulli and her collaborators showed that in mammals a particular group of regulatory proteins, polycomb group (PcG) proteins, maintains transcriptional repression throughout mammalian development, mostly by regulating chromatin structure.[16] One component of this machinery, Polycomb Repressive Complex 2 (PRC2), is responsible for the methylation of histone H3 lysine 27 (H3K27me2/3). Moreover, the authors showed that PRC2 also methylated another protein factor, Jarid2, and that Jarid2 methylation is important to promote PRC2 activity at a locus devoid of H3K27me3 and for the correct deposition of this mark during cell differentiation. I.e., Jarid 2 methylation fine-tunes PRC2 activity depending on the chromatin context. This shows that the methylation of special histone amino acids plays an important role in gene regulation during development as part of a highly complex machinery that is regulated by regulatory proteins that are coded for by DNA.

Though transcriptional activation and demethylation can sometimes be correlated, a causal relationship has not been demonstrated. According to Timothy Bestor et al., the available data do not support "the existence of a biochemical system that regulates embryogenesis by programmed methylation and demethylation of regulatory sequences."[17] The authors suggest that "mammalian genomic methylation patterns represent an evolutionary adaptation of a genome defense system that endows genomes with the ability to inactivate specific genomic regions in a self-perpetuating manner which is essentially irreversible over the lifespan of the organism." The authors thus point to DNA cytosine methylation's crucial involvement in processes such as transposon silencing, imprinting, and X chromosome inactivation.[15,19,17,20]

To summarize this section: Research on chromatin and histone modification as well as on DNA methylation developed separately from one another for about two decades before both began to be labelled epigenetics. Research labelled epigenetics itself originated outside of these research strands, namely in developmental biology.

2. From chromatin research as epigenetics to epigenetics as chromatin research

2.1 The origin of epigenetics in developmental biology

The term "epigenetic" as adjective existed many centuries before the noun "epigenetics" was used; it was related to "epigenesis" and not "epigenetics." The term "epigenesis" was introduced by the physician and physiologist William Harvey around 1650 for the conception of development as a gradual process of increasing complexity from initially homogeneous material in the egg, an

idea that was originally proposed by Aristotle. Epigenesis contrasted with preformation, according to which the embryo or parts of it are preformed from origination. The term *genesis* (gr.) can be translated as origin, and *epi* as on or after.

The history of epigenetics has been widely studied.[21,10,22,8,12] In 1942 embryologist Conrad Waddington introduced the term "epigenetics" into modern biology, emphasizing its relationship to the classical concept of "epigenesis." Waddington conceived of "epigenetics" as the "whole complex of developmental processes" that lie between "genotype and phenotype".[23] His often-cited model of an "epigenetic landscape", illustrating the various developmental pathways a cell might take during differentiation, attributes a major role to the genes which underlie the landscape, acting to structure it. Waddington believed that the presence or absence of particular genes determines the path a cell will follow from a certain point of divergence.[24]

Another conception of "epigenetics" was suggested by microbiologist David Nanney who in microorganisms distinguished between a genetic and an "auxiliatory", epigenetic, control mechanism of gene expression.[25] The latter could lead to different phenotypes of cells with the same genotypes and be perpetuated through cell division. Research that pursued the questions Waddington raised was later called developmental genetics, whereas Nanney's approach was included in research on cellular memory.[26]

Subsequent research on the regulation of gene expression in the development of higher organisms carried out since the 1960s by molecular biologists, was also not labelled epigenetic.[27,28,8] Convinced that models based on specific repressors, which were developed in bacteria, were not applicable to higher organisms, Eric Davidson postulated non-specific inhibition of gene expression in eukaryotes by histones combined with selective activators.[8] In 1969, Roy Britten and Davidson proposed a theoretical model, in which various types of genes at different hierarchical levels of regulation interact to control the fates of cells in early development through differential gene expression.[29] This theory not only contained the first detailed model of gene regulation in higher organisms, but also predicted wide evolutionary implications: Changes in regulatory regions may result in stable systems of genes that could enable evolutionary novelties. Their model, in which the concept of genetic information in the form of DNA sequences was central, was further developed by experimental research on gene regulation in development and by the study of evolutionary mechanisms for the changes of body plans.[9,30]

2.2 DNA methylation as epigenetics

The discovery of imprinted genes in mice and men in the 1990s led to a close association of DNA methylation with epigenetics. But research labelled "epigenetics" remained marginal until heritability was added to its definition. Robin Holliday proposed that patterns of DNA methylation were heritable through cell division and that there were also

examples of epigenetic inheritance through the germ line. Therefore (de-) methylation of DNA should be called epigenetic.[31] A widely used new definition of epigenetics included this aspect: "the study of mitotically and meiotically heritable changes in gene function that cannot be explained by changes in DNA sequences."[32] However, as will be shown later, the idea of transgenerational epigenetic inheritance in higher organisms is highly problematic. Moreover, this definition and other recent definitions do not distinguish between the propagation of modifications through cell division (thus helping to maintain a pattern of gene expression) and other cases in which the modifications are simply part of the transcriptional apparatus that is its biochemistry.[10] From around 2000, modifications of histone proteins, too, were subsumed under "epigenetic inheritance."

3. The current usage of the term epigenetics

At present, the term epigenetics is mainly used without reference to the meaning that Waddington gave it. In biology, it is now used for all chromatin and DNA modifications and other transcription regulators that act in the context of chromatin.[37] This includes research on "histone and DNA-modifying enzymes, nucleosome remodelers, histone chaperones, and chromatin-binding proteins to facilitate transcription factor and polymerase action."[35] Research labelled epigenetics grew rapidly and changed over time from developmental biology to molecular biology and informatics, "making it unsurprising that there is currently room for major differences of opinion about the most appropriate use of this term."[35] In molecular biology, cell biology and chromatin research, epigenetics can relate to research on chromatin structure and function, DNA methylation and its causes, or the study of the self-perpetuation of signals as a requirement for cells to retain memories of past states. Increasingly, epigenetics refers to long non-coding RNA in transcriptional regulation and small interfering RNA as inhibitors of transcription and translation. Moreover, studies labelled epigenetic also look at the interaction of DNA sequence-specific transcription factors, repressors, and RNA polymerases with histone proteins, chromatin compaction, looping, etc. in processes of gene regulation. Mark Ptashne is of the opinion that genetic research on gene regulation should be termed epigenetic instead; according to him, we should understand epigenetic changes as a subset of gene regulatory changes or, following Waddington, "refer to all developmental gene regulation (including signaling) as epigenetic", because development is a process with no essential changes in DNA sequence.[36]

Given the multitude of different definitions and interpretations of epigenetics, some researchers consider the current usage of the term highly problematic, unless it is specified. John Greally urges researchers to clarify the way in which they use the term epigenetics, and he also made it clear what should not be labelled epigenetics: "If we mean epi+genetics—the layer of information beyond the genome—it is unclear why we don't just say 'transcription regulation' instead. If our definition is basically a proxy for

the mediation of environmental responses, this should not be equated with epigenetic processes at all."[37]

Even though molecular and cell biologists in different fields define epigenetics in very different ways, all of them perceive a close relationship between genetics and the mechanisms they study under the label of epigenetics, with borders sometimes blurred. Given the vagueness of the term epigenetics, and the prevalence of chromatin in all these epigenetic studies, we can ask: Wouldn't it be beneficial to rename epigenetics to chromatin research?

4. Some methodological problems with epigenetics studies in humans

Among the biggest confounders of epigenetic studies are genetic variation and cell mixture distribution.[38] Large proportion of differences in DNA methylation between individuals can be attributed to DNA sequence.[35] According to Gertz et al., DNA sequence accounted for up to 80% of the DNA methylation variability and "the majority of variation in DNA methylation can be explained by genotype."[39] They conclude that the genotype will have to be taken into account when assessing DNA methylation in the context of disease. Reverse causation, i.e., the change of DNA methylation as a consequence of transcription, reflecting rather than causing the differences in gene expression, has been observed in a number of cases.[40] Lappalainen and Greally see one of the reasons for the current problems of interpretability of epigenome-wide association studies (EWAS) in the fact that many of these studies do not measure or account for genetic effects on DNA methylation.[35]

Severe methodological flaws were discovered by Marzi et al. when testing the hypothesis that victimization of young people across childhood and adolescence is associated with DNA methylation, among them the disregard of the confounding effect of tobacco smoking - changes in smoking behaviors were linked to changes in DNA methylation[41] - and flawed or non-existing co-twin control tests.[42] Their epidemiological analysis of epigenetic effects of early-life stress did not support the hypothesis of robust changes in DNA methylation in victimized young people. The authors therefore consider it possible that epigenetic epidemiology is not yet well matched to experimental, nonhuman models in uncovering the biological embedding of stress. Pointing to the great methodical problems in social epigenomic studies in general, such as their low statistical power, some sociologists recommend relying instead on genomic methodology: "With the advent and growing robustness of genomic methodologies, sociologists are in an enviable position to adopt these tools and integrate them into their research."[43]

5. Common misconceptions and unsupported speculations about epigenetics

5.1. Epigenetics does not relativize the importance of genes

Some misconceptions related to epigenetics have been recently discussed elsewhere[33] a summary of which is included in this section. Due to the widespread public and social

scientist emphasis on histone modification and DNA methylation in epigenetics, the primary role of transcription factors, other regulatory proteins, such as those of the polycomb group (mentioned in section 1.2.2) and RNA in the transcription process is largely ignored. But, as was shown in section 1.2.2, it is impossible to separate epigenetics from genetics, i.e., DNA sequence-specific events: Because the enzymes that attach modifying molecules to DNA or histones do not recognize specific DNA sequences - every nucleosome (and hence every gene) appears the same to the enzyme -, specific regulatory factors are necessary to target the transcriptional regulatory machinery to specific DNA sequences. Development, for example, is based on the specific turning on and off of sets of genes over time.

Transcription factors also mediate environmental influences on gene activity and maintain cellular memory in a sequence-specific way. In addition, transcription factors are involved in the initial stages of X chromosome inactivation and imprinting, which is then maintained by DNA methylation. Adrian Bird believes that the close interaction between epigenetic marks and genetics is dissolving the distinctiveness of epigenetics. "And I think that's a good thing."[44]

5.2 The environment has no lasting impact on the change of epigenetic marks

Epigenetics has often been used to support claims of a direct impact of environmental factors on hereditary characteristics. Thus, sociologist Maurizio Meloni, based on his understanding of epigenetics, favours a view of "race, heredity, and biology" that depends "on a complex entanglement of environmental and biological factors in which the human body and its genome become a porous and impressionable material, shaped by all sorts of social pressures originating in society at large."[45]

This view is contradicted by the fact that there is no evidence that epigenetics renders organisms more impressionable by the environment. Adrian Bird has repeatedly emphasized that, contrary to what is commonly believed, the environment does not inform the genome through epigenetics, but that it is the DNA sequences in the genome that influence the epigenome: "If you ask people about epigenetics, the first thing they talk about is the environment impacting on the way genes are expressed. And with epigenetics you also have the concept of heritability which offers a way in which you could get environmental information put into the genome and then transmit it. But, in fact, the evidence suggests that the genome is heavily insulated from the environment. ... To me, it seems that for a lot of aspects of genome management, like DNA methylation, the logic is internal to the cell. ... The environment is not informing the genome. If the logic is internal, then the DNA sequence is likely to be impacting on the epigenome. And there's quite a lot of evidence that that's the case."[46]

The environment can act on the phenotype through transcriptional regulation and cellular differentiation, and much of the resulting stability and cellular memory is based on gene

regulatory networks involving feedback loops. According to Bernhard Horsthemke, chromatin modifications are insusceptible to the direct influence of environmental factors, apart from some synthetic inhibitors of chromatin modifying enzymes. Rather, environmental factors affect gene expression through signalling cascades, which activate or repress transcription factors.[38] In the often-cited example of the Agouti mouse, mothers can modulate the coat color of their progeny through a specific diet of methyl donors, but this effect only lasts for two generations, indicating that the influence of diet is not stable or truly transgenerational,[47] (see also section 5.4).

5.3 Diseases related to epigenetic defects are often caused by mutations

Some studies point to changes of cell fate decisions as a response to the deficiency of micronutrients or to endocrine disruptors. Endocrine-disrupting chemicals modify the function of the normal endocrine system and represent a major area of interest in epigenetics research.[35] In a well-studied case, mice that were exposed in utero to certain chemicals (of the organotin family, members of which are used as pesticides) accumulated fat from birth to adulthood. These phenotypic effects appeared to be mediated by receptors that cause mesenchymal stem cells to differentiate preferentially into the adipocyte (fat cell) lineage. This means that they do not require the reprogramming of a specific cell type.[35]

Despite many misconceptions about the mechanisms involved, there is a lot of evidence that epigenetic defects contribute to human disease.[38] According to Horsthemke, primary epimutations, i.e., aberrant chromatin states without DNA sequence changes, are very rare; examples are imprinting defects that cause certain syndromes. However, most epimutations result from genetic mutations. That means that they are not the cause of the disease but part of the mechanism by which a genetic mutation causes disease.[38]

An example are autism spectrum disorders like Rett and Fragile X syndromes that have been shown by Adrian Bird to be caused by mutations.[46,48] The Rett syndrome is caused by mutations in the gene that encodes the methyl CpG binding protein 2 (MECP2) that reads DNA methylation and plays an important role in nerve cells. If it is mutated, the DNA methylation doesn't change, but the protein that reads it is lost. The Fragile X syndrome shows an X linked dominant inheritance. The methylation pattern of certain genes changes, but, as Bird emphasized, "it is caused primarily by a mutation....In both of these conditions, the DNA sequence changes, and then epigenetics is involved downstream of that."[46]

Another example is the changing of fur colour and body weight in offspring of pregnant Agouti mice that had been fed with folic acid as methyl donors; the assumption was that this happened via increased CpG methylation of a transposable element in the *agouti* gene in mice and downregulation of *agouti* transcription. Some epigeneticists have used this as support for the hypothesis about

intrauterine programming of adult disease.[38] However, it is also possible that the increase in methylation at this locus is a secondary effect caused by downregulation of *agouti* transcription after methyl donor supplementation.[49]

5.4 Transgenerational epigenetic inheritance is questionable in humans

Transgenerational inheritance of acquired traits exists in nematodes through RNA and in plants through methylation. In contrast, proof that transgenerational inheritance has an epigenetic basis in mammals is rare.[50] The function of transgenerational epigenetic inheritance in plants is unclear, and there is no evidence that the inherited traits are adaptive. It is usually associated with transposable elements, viruses, or transgenes and might be, as was suggested for mammals, a byproduct of germline defense strategies.[50] Epigenetics has been invoked in recent years to rehabilitate the pseudoscientific experiments on vernalization by agronomist Trofim Lysenko, a protégé of Stalin,[51] but it was shown that the memory of vernalization is not retained in the next generation, because it is robustly reset in the germline and early embryo.[51]

Transgenerational epigenetic inheritance has been most reliably demonstrated in the nematode C. elegans, where small RNAs can enter the germline and mediate heritable transcriptional silencing in subsequent generations (nematodes do not methylate their genomes). An example is the transgenerational inheritance over many generations of small interfering RNAs that target genes that are relevant for the worm's chemotaxis, nutrition, or virus genome silencing.[52] The small RNAs are transcribed and, unlike methyl groups, contain genetic information. For this reason, and because of the lack of their proven adaptiveness hitherto, the results do not support Lamarck's idea of the inheritance of an acquired trait (see section 5.5). Results in nematodes cannot easily be applied to humans. Nematodes have a very short generation time and unlike higher animals possess RNA-dependent RNA polymerases that can copy small RNA molecules for many generations. In addition, unlike in C. elegans, most of the alleged transgenerationally inherited traits in humans, such as the effects of starvation, are detrimental.

In general, many of the suggested examples of epigenetic inheritance in humans concern inter- rather than transgenerational effects and rarely exclude DNA sequence changes as the underlying cause for heritability. Parental or intergenerational effects occur when the uterus is exposed to toxins, viruses, detrimental nutritional, or hormonal environments that directly affect the developing embryo and its germline. This exposure usually impacts the first generation, but occasionally also grandchildren. In contrast, transgenerational effects relate to generations that were not exposed to the initial environmental trigger, i.e., to great-grandchildren and beyond. Intergenerational effects occur in humans and other mammals, but there are two rounds of efficient reprogramming and erasure of DNA methylation in the development of totipotent

cells in the early embryo as well as during germ cell differentiation. It is widely believed that this reprogramming prevents the inheritance of most of the epigenetic marks, though some gene loci escape it. According to Edith Heard and Robert Martienssen "although much attention has been drawn to the potential implications of transgenerational inheritance for human health, so far there is little support."[50]

The dynamics of demethylation and remethylation during early development seem to be more complex than previously assumed. Edwards et al. showed that the large majority of CpG island promoters are not subject to these waves of methylation and demethylation because they are unmethylated at all stages. The sex-specific methylation at imprinting control regions is demethylated only in the first round, whereas the small population of young, CpG-rich transposons largely escapes both rounds of demethylation. Only sequences that appear to have little evidence of biological function, such as old and inactive transposon remnants, satellite, and other repeated DNA, undergo the double wave of demethylation and remethylation.[20] The authors also showed that genomic methylation patterns at regulatory sequences are essentially static during development, and that the demethylation of promoters upon transcriptional activation is likely a consequence rather than a cause of the activation. The question of whether and to what extent DNA methylation is involved in gene regulation has not yet been finally clarified.

According to Bernhard Horsthemke, most of the studies that claim to have demonstrated transgenerational epigenetic inheritance through DNA methylation or sperm RNA - studies that showed responses to environmental metabolic factors (high-fat diet, obesity, diabetes, undernourishment, and trauma) in mice and rats—still await independent confirmation.[53] It is very difficult, Horsthemke says, to provide conclusive proof for transgenerational epigenetic inheritance in mammals, especially humans, because its study is confounded by genetic inheritance and the impacts of ecology and culture. Some studies, such as those on the transgenerational effects of endocrine disruptors and high-fat diet on the DNA methylome, have been challenged by others.

A key study about the allegedly long-lasting effects of endocrine disruptors reported that the exposure of pregnant female rats to the endocrine disruptor vinclozolin affected male fertility in subsequent generations and that it was associated with epigenetic changes in the germline.[54] This interpretation was refuted by Iqbal et al. who showed conclusively that these epigenetic changes are corrected by germline reprogramming events in the next generation.[55] Emma Whitelaw pointed to the fact that the evidence of epigenetic effects lasting for more than one generation as purported in studies on transgenerational effects of the Dutch hunger winter and of PTSD after the world trade center attacks[56,57] has been inconclusive; she also observed that studies refuting this idea were mainly absent from the literature: "It is very difficult to publish negative results, no matter how important those negative results might be."

As a result, the positive studies "seem to be uncontested to those outside the field."[58]

The maternal environment can have long-lasting effects on our health. In the Dutch hunger winter, for example, severe undernourishment affected pregnant women, their unborn offspring, and the offspring's fetal germ cells. But the increased incidence of cardiovascular and metabolic disease observed in the first generation is not due to the transmission of epigenetic information through the maternal germline, but a direct consequence of the exposure in the uterus, a phenomenon called "fetal programming" or - if fetal germ cells and the second generation are affected - "intergenerational inheritance."[53]

5.5 Epigenetics does not vindicate Lamarckism

Statements about epigenetics rehabilitating so-called Lamarckian inheritance are common in scientific publications and in the popular press. But even if there was incontrovertible evidence for the transmission across generations of epigenetic modifications, this should not count as a vindication of "Lamarckian inheritance." Jean-Baptiste Lamarck (1744-1829) was a renowned naturalist because of his work in botany and zoology and his introduction of the term "biologie." His theory of organismal change over long periods of time, which he put forward around 1800, was the first comprehensive theory of organic evolution. The theory aimed at explaining the diversity of forms and the graded series of animals' perfection, which he, intellectually committed to Leibniz, assessed in terms of their complexity. Animals' power of progressively complex organization and their capacity to react to the special conditions of the environment in order to stay in harmony with it (adaption) were the causes for evolutionary change. This implied the ancient ideas that organs are strengthened and enlarged by use and weakened and diminished by disuse and that these actively acquired characteristics are inherited. But, as historians have long stressed, this idea was not invented by Lamarck, but so universally accepted since ancient times (supported for example by Aristotle) that Lamarck did not elaborate on it. Until the early twentieth century, it was adopted by most naturalists, including by Charles Darwin, who supplemented but not replaced it with his theory of natural selection. (Darwin, however, did not support the idea of a drive towards greater perfection.) Lamarck's theory also included the ancient idea of spontaneous generation of lower organisms from non-living material, which accounted for the continued existence of primitive organisms.

The idea of the inheritance of acquired characteristics as a means for adaptation was first rejected in an influential way by August Weismann in 1883. This rejection gave rise to Weismannian neo-Darwinism in late 19th century, in which Darwinism was stripped of "Lamarckism." The idea of the inheritance of actively acquired characteristics as a means for adaptation was abandoned by most biologists in the 1920s and 30s with the advent of population genetics, a synthesis of Mendelian genetics and evolutionary theory. From this perspective, "Lamarckism" should

be a label for the belief that environmentally induced inherited variations if they were actively acquired and adaptive, can have a long-lasting impact on evolution. However, the phenomena of inherited variation related to epigenetic marks do not belong to this category for three reasons: first, they are not actively acquired; second, they are not adaptive (except by chance), and in most cases even detrimental; and third, they are not stable over many generations and so do not have a long-lasting impact on evolution.

6. Concluding remarks - Epigenetics is not a paradigm shift in understanding heredity

Most researchers in developmental, cell, and molecular biology greatly diverge in their understanding and definitions of epigenetics. They do not question the fact that the fundamental characteristics of organisms, such as their species and individual specificity in molecular composition, morphology, and body architecture, are primarily based on the genome and generated through the control of their early development by gene regulatory networks. Genes do not determine every single trait, but the reaction norm, i.e., the range of potential - reversible - phenotypic variations in different environments. In contrast, social scientists and the public press largely understand epigenetics as alleged environmentally caused and transgenerationally inherited DNA methylation. However, most of the reported effects, such as those of trauma and nutritional deficiencies, are in fact results of the direct exposure of the mother, the embryo, and sometimes the embryo's germ cells to the adverse conditions.

The rise of epigenetics has been accompanied by some of its proponents', and, later, social scientists' and the popular press's far-reaching claims and revolutionary attitudes towards well-established knowledge. Florian Maderspacher, then an editor of the journal Current Biology, in 2010 introduced the term "epigenetics hype" to describe the widespread revolutionary claims of a "victory over genes" by epigenetics in scientific and popular literature.[59] They were, for example, expressed in the assertion that "DNA Is Not Destiny ...The new science of epigenetics rewrites the rules of disease, heredity, and identity" (https://www.discovermagazine.com/the-sciences/dna-is-not-destiny-the-new-science-of-epigenetics). Meloni is of the opinion that "recent findings in postgenomic biology, with epigenetics as a key-case, look very much like a paradigm-shift, or at a minimum, a 'profound disturbance' of the dominant understanding of biological heredity."[45]

However, as the above has shown, epigenetics cannot be clearly separated from genetics because epigenetic mechanisms such as DNA methylation are dependent on DNA sequence specific events. Moreover, DNA methylation does not silence active promoters of genes but affects genes that are already silent. It is also generally accepted that DNA methylation plays a major role in processes like transposon silencing, imprinting, and X chromosome inactivation, its role in gene regulation in development has not yet been finally resolved. Gene activation and repression during development are controlled not by DNA methylation but by

well-established protein- and RNA- based mechanisms.

To summarize, epigenetics, a field with revolutionary aspirations, did not bring about a revolution or paradigm shift (Thomas Kuhn)[60] in biology; research on chromatin modification, DNA methylation, etc. did not replace genetic and genomic research. The various strands of epigenetic or chromatin research are a continuation of research related to the old question of gene regulation in the development of higher organisms, including novel concepts and techniques. Epigenetics, however defined, has led to the opening up of new directions of basic research and of medical and other applications, but does not call into question the paradigm of genomic information as a major cause of heredity and development.

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