

Review

Epigenetics: It's Getting Old. Past Meets Future in Paleoepigenetics

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Recent years have witnessed the rise of ancient DNA (aDNA) technology, allowing comparative genomics to be carried out at unprecedented time resolution. While it is relatively straightforward to use aDNA to identify recent genomic changes, it is much less clear how to utilize it to study changes in epigenetic regulation. Here we review recent works demonstrating that highly degraded aDNA still contains sufficient information to allow reconstruction of epigenetic signals, including DNA methylation and nucleosome positioning maps. We discuss challenges arising from the tissue specificity of epigenetics, and show how some of them might in fact turn into advantages. Finally, we introduce a method to infer methylation states in tissues that do not tend to be preserved over time.

Trends

How are ancient epigenomes reconstructed?

Which epigenetic layers could be reconstructed?

The pros and cons of tissue specificity in paleoepigenetics.

A novel method to infer methylation in unobtainable tissues.

Unearthing Epigenetic Layers

The epigenome is viewed today as a collection of regulatory layers that control when, where, and how genes are turned on and off. These layers are passed through cellular or organismal generations and include modifications to the DNA (i.e., DNA methylation) and to the proteins that package it (e.g., histone modifications), as well as regulation by noncoding RNAs (e.g., miRNAs) and changes in the 3D conformation of the genome. While it is still debated which layers are epigenetic and to what extent they are heritable (Box 1) [1–3], it is nevertheless accepted that alterations in regulatory layers can propel substantial phenotypic changes [4]. Such alterations can stem from sequence mutations, but also from environmental factors, or simply be a result of stochastic processes [5]. This combination of plasticity and heredity led to the growing recognition that epigenetic evolution occurs in short timescales, precedes sequence adaptation [6], and could underlie phenotypic differences between closely related species [6–11]. In light of this, studying recent adaptations of a species requires a comparison of epigenomes of close evolutionary relatives [12–14].

Unfortunately, very often, the extant sister group of a species is deeply diverged from it, allowing only crude resolution in determining the timing of evolutionary events. For example, the closest extant relatives of humans are the chimpanzee and the bonobo, from which we diverged ~5–8 million years ago [15–17]. As a result, it is usually impossible to determine whether an evolutionary change along our lineage happened recently and is unique to modern humans, or whether it occurred in our deep past, at times when our ancestors displayed many ancestral properties such as a brain the size of a chimpanzee's. However, exciting developments in the rising field of ancient DNA (aDNA) provide access to genomes of extinct species, and thus pave the way for much finer temporal analyses.

The Rise of Ancient Genomics

Recent years have witnessed the successful high-quality sequencing of two individuals from archaic human groups – a Denisovan at 30× coverage [18], and a Neanderthal at 52× coverage

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Box 1. The Different Perceptions of Epigenetics

The term 'epigenetics' was coined by Conrad Waddington [84] to describe the interactions between genes and their products to produce phenotypes. This incredible insight was published in 1942, in the pre-gene era. Nowadays, the term 'epigenetics' is used in different ways. The narrower and more traditional definition is a 'stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence' [85]. The broader everyday definition refers to the complete set of regulatory layers that have the potential of being inherited, whether genetically driven or non-genetically driven. According to this definition, any change to DNA methylation, histone modifications, chromatin packaging, small RNA, etc. is considered part of the epigenome. In this review, we refer to epigenetics in its broader definition.

[19]. These works provided the full DNA sequence of these archaic humans, allowing in some cases to associate phenotypes with genetic differences [19,20]. However, as noted earlier, genetics alone cannot explain the full spectrum of phenotypic adaptations. These ancient genomes revealed that the number of fixed amino acid substitutions that separate present-day humans from archaic ones stands at only 96 (in 87 proteins) [19]. This relatively small number stems from the fact that we share a very recent common ancestor with these archaic humans, around 550 000–765 000 years ago [19]. Such recent divergence was too young for the accumulation of many amino acid changes, but sufficient to give rise to numerous noncoding sequence changes of potentially regulatory roles. However, our ability to predict the regulatory effect of a sequence change is very limited, and it is therefore necessary to develop ways to map epigenetic layers in aDNA. On first glimpse, it might look like an insurmountable endeavor; aDNA is broken and degraded, and thus inherently inactive and includes little to no remnants of cellular context. Moreover, while some extracellular proteins survive for periods of time exceeding those of DNA, most proteins rapidly break down, leaving little to no trace of their activity patterns in the premortem cell [21–23]. Nevertheless, recent works demonstrated that at least some of the epigenetic signals might be accurately reconstructed [24–26], thus providing information that could not have been gained from genetics alone [27,28]. In this review, we describe these recent developments and discuss future possibilities in this novel field, which we refer to as 'paleoepigenetics'.

Reconstructing Archaic DNA Methylation Maps

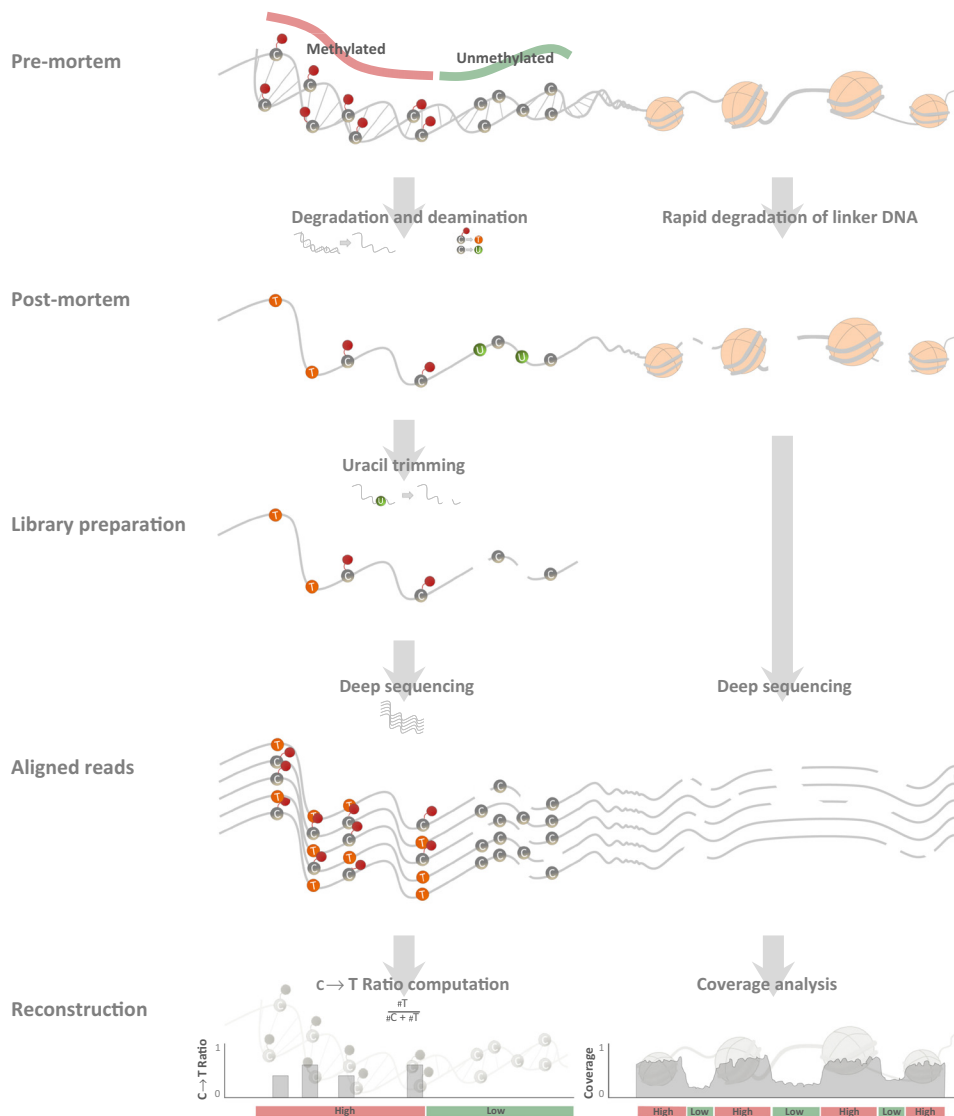
DNA methylation, the conversion of cytosine to 5'-methyl-cytosine by DNA methyltransferases, is a fundamental epigenetic mark, involved in the regulation of gene activity. In mammals, methylation usually occurs in the context of a cytosine followed by a guanine (CpG). We are still far from predicting the effect of a local change in methylation on the expression level of a gene. The strongest predictive power is in promoter regions, where hypermethylation is associated with gene silencing [29]. Several recent works demonstrated that premortem DNA methylation patterns can be reconstructed from aDNA sequences, either by direct measurement or by computational algorithms (Table 1). Direct measurement employs protocols that are regularly used in modern samples (e.g., bisulfite conversion or methyl-enrichment methods, followed by sequencing [30,31]). These methods can produce single nucleotide resolution maps, but their applicability to aDNA depends on several factors. Bisulfite sequencing (BS-seq) involves the conversion of unmethylated cytosines into uracils, followed by sequencing, and therefore requires special allocation of DNA, as this conversion does not allow the reuse of the same sample in the future (Table 1). This makes rare samples, or samples with minute quantities of DNA, not suitable for BS-seq. Enrichment-based methods, by contrast, are based on the precipitation of methylated cytosines, and thus generally do not modify the DNA. However, these methods are inherently biased towards CpG-rich regions and long fragments of DNA [30,32]. Finally, both methods will measure systematically skewed levels of methylation in samples that have gone through elevated levels of degradation, that is, the spontaneous hydrolytic deamination of methylated cytosines into thymines [30,32]. Altogether, these are the methods of choice for typically younger, better preserved and relatively abundant aDNA samples (Table 1).

Table 1. A Comparative Summary of the Methods That Have Been Used to Date to Measure DNA Methylation in Ancient DNA

Method	Sample Quality Required	Additional Use of Sample after Sequencing	Timescale	Resolution	Ancient Samples on which the Method Was Implemented	Refs
Bisulfite-sequencing	Preferentially less damaged	Yes	Generally younger samples	Single nucleotide resolution	Bison, barley, human	[33–35]
Methyl-enrichment followed by sequencing	Limited to samples with low fragmentation and deamination	Yes	Generally younger samples	Regional	Mammoth, human, polar bear, several equid species	[32]
Deamination-based computational reconstruction	Requires high-coverage samples	No	Generally older samples	Regional. Higher resolution the higher the coverage and deamination	Human (Paleo-Eskimo, Neanderthal and Denisovan)	[24,26]

The first direct measurement of ancient methylation was done in a 26 000-year-old Pleistocene bison, which was preserved in permafrost, thus allowing exceptional preservation of methylation [33]. Later works included the investigation of DNA methylation in 30 Native American skeletal remains, ranging from 230 to 4500 years before present [34], and the analysis of methylation patterns in virus-infected archeological barley from Egypt, which revealed epigenetic response to ancient viral infection [35]. Taken together, these works have shown that when aDNA is preserved under favorable conditions, methylation can survive for tens of thousands of years, whereas in less favorable conditions such as warmer regions, its half-life can be as short as ~1500 years [33,35].

When direct measurement is less suitable, a purely computational strategy can be employed. The computational reconstruction of methylation maps is made possible owing to the natural degradation processes of DNA [25]. Remarkably, whereas DNA degradation is a major obstacle in aDNA sequencing, it is an information-rich and a key resource in epigenetic reconstruction. The reconstruction method harnesses the fact that a predominant degradation process of aDNA, the deamination of cytosines, affects methylated and unmethylated cytosines differently: whereas methylated cytosines are deaminated into thymines, unmethylated cytosines become uracils (Figure 1). Protocols for processing aDNA frequently include a uracil removal step or a polymerase that does not replicate through uracils, resulting in an asymmetry between the deamination products of methylated and unmethylated cytosines [25]. This asymmetry can be utilized to infer the premortem methylation status of the original cytosine by computing the fraction of C's that became T's in each CpG position; positions that exhibit a high C→T ratio represent positions with high premortem methylation levels, whereas positions with a low C→T ratio suggest that deamination events were all C→U, and hence the position was unmethylated premortem (Figure 1). This method was used to reconstruct methylation data for a ~4000-year-old Saqqaq Paleo-Eskimo from Greenland [24], as well the full methylomes of the Neanderthal and the Denisovan [26]. Compared with classic methylation mapping protocols, this method has two key disadvantages: (i) it can only be applied to high-coverage ancient genomes, and (ii) it uses a sliding window to cope with low levels of deamination, which results in a regional, rather than base pair, methylation map. The higher are the coverage and the deamination rate, the shorter is the window that should be used. By contrast, since it builds upon DNA degradation it is expected to be more accurate the older and the more degraded the DNA is. Thus, it is the



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Figure 1. The Processes that Allow the Reconstruction of DNA Methylation and Nucleosome Positioning Maps. The left panel shows a DNA section with a methylated locus and an unmethylated locus. After death, the DNA is gradually degraded into shorter fragments and at the same time, cytosines are going through a hydrolytic process of deamination, which turns methylated C's into T's, and unmethylated C's into U's. After excavation, ancient DNA is treated with enzymes that remove U's, but not T's. As a result, regions that were methylated pre-mortem contain many T's in C positions, whereas regions that were unmethylated contain mainly C's in C positions. C→T ratio is computed for each position, reflecting the pre-mortem levels of methylation [24–26]. The right panel depicts nucleosomes, made up of DNA wrapped around histone proteins. Linker DNA between adjacent nucleosomes is degraded faster postmortem. Thus, when the DNA is sequenced, these regions exhibit lower coverage [24].

method of choice for highly degraded samples, at least as long as they can still be sequenced to high coverage (Table 1).

To date, extinct and extant Homo groups drew particular attention in aDNA sequencing. However, other species have not been overlooked; many genomes of ancient organisms have been partially or fully sequenced. Examples include the cave bear [36], moa [37,38], mastodon [39], ground sloth [40], dog [41], woolly rhino [42], cave hyena [43], steppe bison [44], polar bear

[45], Adelie penguin [46], giant deer [47], and even a 560–780-thousand-year-old horse [48]. The relatively low quality of these genomes makes epigenetic reconstruction essentially impossible, but promising new high-coverage genomes, such as those of woolly mammoths [49,50] and horses [51], will enable paleoepigenetic reconstruction in other species in the near future.

Differentially Methylated Regions

The reconstructed Denisovan and Neanderthal methylation maps provided a rare opportunity to gain insight into the epigenetic history of humans. Comparing these archaic maps to the bone DNA methylation maps of present-day humans revealed ~2000 differentially methylated regions (DMRs) [26]. A notable example is the five DMRs in the promoters, gene bodies, and enhancers of the *HOXD* cluster region, which were suggested to mark regulatory changes that lie behind many of the differences in limb morphology between archaic and present-day humans, including shorter and more curved limbs, broader joints, and more robust hands [26,52]. Notably, the related *HOXD* genes show no changes at the protein level, suggesting that these phenotypes directly arise from changes in gene activity levels [26]. Further examination of genes, whose methylation pattern had changed along the *Homo sapiens* lineage, revealed that many of them are expressed in the brain, and that they tend to be linked to diseases, and especially to neurological and psychiatric disorders [26]. This suggests a tantalizing trade-off scenario in which recent changes in the activity of our genes might have been advantageous in some aspects, but at the same time might have also given rise to diseases. This can be a result of the fact that these changes are relatively recent and thus might not have had sufficient time to fully adapt to all genetic and environmental backgrounds.

Tissue-Specificity as an Obstacle and a Tool

Detecting DMRs

The differentiation of tissues is concomitant with and driven by epigenetic changes. Therefore, the DNA methylation maps of bone cells differ from those of other tissues. As a result, a DMR found when comparing the bones of two individuals would not necessarily exist when comparing these individuals' brains, for example. However, if a comparison of bone methylation maps revealed a DMR in a region that shows very little variability across tissues, then it is also likely to hold in non-bone archaic tissues. This is expected to be the case in the majority of the genome, as ~75–80% of DNA methylation is stable across tissues [53,54]. This principle was demonstrated in a study of humans and great apes, where many DMRs that were found in a human–chimpanzee comparison of blood cells were also shown to exist in kidney, heart, and liver cells [13]. Similarly, a study that analyzed DMRs that segregate between human populations reported that DMRs identified in lymphoblastoids tend to be also found in the brain [55]. In general, and similarly to expression patterns [56], the closer two cell types are, the more similar are their patterns of methylation [53,57–59]. In conclusion, although paleoepigenetic studies are confined to the few tissue types that tend to be preserved particularly well (e.g., bone, teeth, and hair), many of the DMRs that are detected in these studies are in all probability relevant to other tissues as well.

A Novel Approach to Overcome Tissue Scarcity

The limited repertoire of ancient tissues is possibly the biggest obstacle in paleoepigenetics. However, exciting new findings from highly preserved mammoths show that DNA can be successfully extracted and sequenced from soft tissues, such as liver, heart, skeletal muscle, and skin as old as 45 000 years [32,49]. Nevertheless, this example is the exception, and bone, teeth, and hair will probably continue to be the main sources of aDNA samples.

To try and overcome the limited repertoire of ancient tissues, we present a novel method based on parsimony reasoning that in some instances will enable to infer the pattern of methylation in tissues that we are very unlikely to ever obtain. As an example, let us consider a DMR that was



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Figure 2. Methylation State Can Be Deduced in Unobtainable Tissues of Ancient Individuals. Each tissue is colored according to the methylation state of the investigated locus, either green (low methylation) or red (high methylation). (A) A locus that is methylated in all present-day human tissues and in all non-bone chimpanzee tissues. Parsimony considerations lead to the conclusion that the ancestral state of this locus was unmethylated in bone and methylated in other tissues, and that there was a bone-specific change along the present-day human lineage. Therefore, this locus is predicted to be methylated in other archaic tissues, such as the Denisovan brain. (B) A similar locus, with a difference that the additional chimpanzee tissues are unmethylated. In this case, parsimony lead to the conclusion that the ancestral locus was probably unmethylated across all tissues, and that there was an elementary change across all present-day human tissues. Such a drastic change can be a result of an epigenetic alteration in early developmental stages or in the germline. Alternatively, this could result from a change in the activity of a regulatory factor that is active across tissues. In this scenario, the most probable prediction is that this locus is unmethylated in the archaic tissues.

found in a comparison of bones of present-day and archaic humans. In this example, the locus in present-day humans is methylated, whereas in both the Denisovan and the Neanderthal it is unmethylated (Figure 2). With this information alone, we are unable to determine the methylation state of this locus in other Denisovan tissues, such as the brain. But, an addition of an outgroup (e.g., chimpanzee) and other tissue types in extant species might change the picture. For example, if the locus is unmethylated in the chimpanzee bone, but is methylated in the brains of present-day humans and chimpanzees, then the parsimonious scenario is a bone-specific epimutation that happened on the present-day human lineage and changed the locus from being unmethylated to being methylated. In this case, the conclusion is that this locus was methylated in the Denisovan brain (Figure 2A). Under a different scenario, assuming the same levels of methylation in bones as above, but low methylation levels in the additional tissues, we

would end up with the conclusion that the ancestral state of the locus was unmethylated in all tissues, and that an elementary change along the modern human lineage affected all tissues, switching the locus from being unmethylated to being methylated. Such an elementary change might occur if the epigenetic state of a locus is determined shortly after conception and before tissue differentiation, or if methylation in the locus is controlled by a protein that regulates methylation in many different tissues. In this example, the parsimonious conclusion would be that the locus was unmethylated in the Denisovan brain (Figure 2B).

Parsimony reasoning has a solid statistical ground when the probability of an evolutionary event within a studied time window is low [60]. Consequently, comparative genomics must take into account the rate of mutations in the examined locus; fast evolving loci (e.g., mitochondrial DNA and microsatellites) are generally preferable for populations and close species, whereas the ultraconserved ribosomal genes are suitable for comparisons of distantly related species. Similar considerations should be taken into account in comparative epigenomics. The overall rate of epimutations is still under debate, but several studies showed that the rate per nucleotide position is not much faster than sequence mutations [8,13,26]. For example, only ~2% of positions were shown to be differentially methylated between humans and chimpanzees [13], and less than 1% between archaic and present-day humans [26]. Additionally, it was shown that the rate of sequence and methylation changes are correlated, that is, conserved genes exhibit lower levels of epimutations [13,26,61]. Therefore, epimutation rate and the evolutionary distance between the analyzed species should dictate on which loci the above method could be applied.

In conclusion, to understand the genetics of an individual organism, a single good sample suffices, regardless of the tissue it came from. However, to understand the epigenetics of an organism, one needs an assortment of tissues. The approach we have presented here provides a glimpse into methylation in tissues that are unlikely to be found in archaeological sites.

Using Tissue-Specificity to Detect Contamination

Another big challenge in aDNA studies is contamination, whether originating from bacteria or from individuals who handled the samples. In a bone sample, any modern human contamination is unlikely to come from bone cells, but rather from tissues such as skin, hair, and the saliva of the researchers who handle the sample. Therefore, examining methylation levels in bone-specific loci should point to the source of the DNA. This principle was used to determine whether aDNA from bones is a remnant of bone cells or rather of blood cells that might also be present in a bone sample. Indeed, the Denisovan and Neanderthal methylation maps were shown to cluster closest to modern bone methylation maps, strongly arguing that these cells are the main source of DNA in ancient bones [26]. Although initially used to establish the reliability of the reconstruction method, a similar approach was used for the Saqqaq Paleo-Eskimo, where DNA was extracted from hair shafts and the reconstructed methylation map was shown to cluster closest to modern hair tissue [24].

Age Determination

As of today, the biological age of an excavated individual is usually approximated through the analysis of its bone morphology, as the skeleton continues to change in shape, size, and composition from birth to adulthood [62]. In samples other than bone, such as the hair tuft used for the sequencing of the Saqqaq individual [63], the task of age determination is far more challenging. However, recent studies have shown that DNA methylation could be utilized for this task, as the methylation status of many genomic positions is highly correlated with biological age [64–66]. Strikingly, as little as five CpGs were sufficient to predict an individual's age with a deviation of approximately 11 years [65]. Later algorithms were based on thousands of additional samples, and achieved even more accurate results on a variety of tissues, including

bone [64–66]. Interestingly, some of these clock CpGs are tissue-independent, and thus the algorithm can probably be applied on samples that comprise a mixture of cell types. Two of the clock CpGs were used to estimate the age of the Paleo-Eskimo, concluding that he was in his fifties at the time of death [24]. Importantly, it was shown that the positions that are age-related in humans can also accurately predict age in chimpanzees, suggesting that the age-related mechanisms are conserved and could probably be applied to other hominins [64].

The Many Facets of Paleoeugenetics

DNA Packaging

Over the years, the field of epigenetics has paid much attention to DNA methylation, but gene regulation is a multifaceted process, comprising additional important layers. One of the key epigenetic layers is the pattern of DNA packaging; eukaryotic DNA is wrapped around a core octamer of histone proteins which, together with the wrapped DNA, is called the nucleosome. Unwrapped DNA between consecutive nucleosomes is called linker DNA. The nucleosome positions along the genome play an important role in gene regulation through the occlusion of DNA-binding proteins from access to the DNA [67]. The histones at the core of the nucleosome probably do not survive much after cell death [21–23], but just enough time to affect DNA fragmentation, whereby DNA tends to break at linker regions faster than at nucleosomal regions [20,24]. The resulting fragmentation signature might be captured by looking at the variation in sequencing coverage, which peaks at nucleosomal DNA (Figure 1). Such an analysis had been carried out for the Paleo-Eskimo sample. The results showed a striking 200 bp periodicity in sequence coverage that matches the typical distance between consecutive nucleosomes. Interestingly, similar periodicities were found in a variety of tissues spanning a wide time range, from a 100-year-old Aboriginal Australian to a 110 000–130 000-year-old polar bear [24]. At the same time, many other aDNA samples do not exhibit such patterns [20], thus the factors that contribute to postmortem nucleosome signatures are still to be deciphered.

Transcription Factor Binding Sites

Transcription factor binding sites (TFBS) are short cis-regulatory elements that are recognized by transcription factors (TFs). Some binding motifs have high predictive power, and mutations within them can be readily interpreted. This information has been used to predict dozens of single nucleotide changes that potentially affected TFBS of Neanderthal and Denisovan genes [18,19]. In some cases, it was shown experimentally how a derived TFBS changed the expression of a nearby gene in present-day humans [68]. A particularly interesting example was found within the *FOXP2* (GenBank accession ID: NM_001172767), a gene that was linked to impairments in language and speech development [69]. Maricic *et al.* found a substitution in intron 8 that is shared by nearly all present-day humans, but polymorphic or completely absent in archaic humans. This substitution is found within the binding site of the transcription factor POU3F2, and was shown experimentally to reduce the activation efficiency of *FOXP2* transcription [70]. Interestingly, the substitution is found within a region of a recent selective sweep, and thus it might have conferred a selective advantage in language acquisition to the humans who have borne it [70].

It is not only mutations in TFBS that can affect gene regulation but also changes in the abundance of a TF [29,71]. The binding of TFs can alter regional DNA methylation levels, and therefore changes in the expression of a TF-coding gene could introduce changes in methylation [29,71]. Hence, such TFs can be identified by looking for methylation changes in both the TF gene and its target genes. This strategy was used to find four TFs whose activity level had significantly changed during the very recent evolution of humans. Among them is the *MEIS1*, a regulator of limb development, which forms complexes with *HOXD* genes, and therefore might have been the driving force behind the many DMRs observed in the *HOXD* cluster [72]. Overall, many of the DMRs observed between archaic and modern humans might actually stem from just

a few TFs that changed their activity levels [26]. Such a model, where changes in a handful of TFs drove DNA methylation changes in hundreds of genes, suggests a burst-like, otherwise known as ‘saltational’, model of evolution, and might explain some of the morphological leaps observed in different lineages [4].

miRNAs

Most mammalian genes are regulated by miRNAs, which are small noncoding RNAs of approximately 22 bases in length [73]. miRNAs affect gene products through multiple interactions, whereby a single miRNA can regulate dozens and even hundreds of different target genes, and a single gene can be regulated by many miRNAs [74]. The binding of a miRNA to its targets is mediated through the base pairing of seven nucleotides which form its seed region. Thus, substitutions in the seed region of a miRNA change its target specificity. Lopez-Valenzuela *et al.* used this observation to identify an ancestral *miR-1304* allele that is found in the Neanderthal but is very rare among present-day humans. The derived allele was predicted to bind at least 10-fold more targets, to be associated with behavior and nervous system development, and was suggested to be a driver of differences in dentition between Neanderthals and present-day humans [75].

miRNAs are in themselves tightly regulated genes, and changes in their activity can be identified not only through changes in the seed sequence but also through changes in regulatory sequences that control their expression levels. Such a change was identified in a putative enhancer of *miR-34c-5p*, which shows human-specific brain expression, and was shown to be under positive selection after the split from Neanderthals [76]. As more and more species-specific miRNAs are identified and their function unraveled, ancient genomes will become central in elucidating the phenotypic effects of miRNA-mediated traits, and the time frame in which these traits were gained [75–79].

Concluding Remarks: One Small Step for a Gene, One Giant Leap for the Organism

Recent evolutionary history is replete with transient, yet dramatic events. For humans, this includes the exodus from Africa, colonization of all climate zones, and the transition to agriculture. Other organisms experienced mass extinctions, drastic climate shifts, and rapid domestication by humans. The ability to infer gene regulation from high-quality DNA of extinct organisms provides a unique opportunity to follow the footsteps of these processes in unprecedented time resolution. In this review, we covered several novel methods that provide means to explore these processes. However, paleoepigenetics is still in its infancy, lacking both in depth and in breadth; important epigenetic layers, such as histone modifications and the 3D conformation of the genome, remain currently out of reach, and the majority of high-quality genomes still come from humans. Nevertheless, borders in this field continue to be pushed back, with aDNA being successfully sequenced from earlier time periods [48], a wider range of tissues [32], and a broader spectrum of climates [35,80–83]. These advances pave the way to venture into new grounds in the study of epigenetics and its role in evolution (see Outstanding Questions).

Acknowledgment

The work was supported by the Israel Science Foundation FIRST individual grant (ISF 1430/13 to L.C. and E.M.); the Israel Science Foundation (ISF 657/12 to E.M.); and the European Research Council (ERC-281781 to E.M.).

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Outstanding Questions

What role does epigenetics play in adaptation and speciation? What fraction of interspecies phenotypic differences can be explained by epigenetics?

Can paleoepigenetics bring mammoths back to life? What role would paleoepigenetics play in de-extinction projects?

What is the rate of epimutations and how does it compare to genetic mutations?

In what ways does the environment affect epimutations? What other factors affect it?

What is the relationship between epigenetic and sequence adaptation?

What are the selection regimes that act on epimutations?

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