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Epigenomics—Technologies and Applications

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Abstract

The advent of high throughput epigenome mapping technologies has ushered in a new era of multi-omics where powerful tools can now delineate and record different layers of genomic output. Integrating various components of the epigenome from these multi-omics measurements allows the interrogation of cellular heterogeneity in addition to the discovery of molecular connectivity maps between the genome and its functional output. Mapping of chromatin accessibility dynamics and higher order chromatin structure has enabled new levels of understanding of cell fate decisions, identity, and function in normal development, physiology, and disease. We provide a perspective on the progress of the epigenomics field and applications, and anticipate an even greater revolution in our understanding of the human epigenome for years to come.

Keywords

epigenetics; gene regulation; genomics; chromatin architecture

Subject Terms

Genetics; Epigenetics

Cells in multicellular organisms are genetically homogeneous but structurally and functionally heterogeneous owing to the differential expression of genes. Classic Mendelian inheritance of phenotypic traits results from allelic differences caused by mutations of the DNA sequence. In contrast, other genetic phenomena, such as X chromosome inactivation during early embryo development in female mammals, position-effect variegation in flies, and chromosomal imprinting, exhibit non-Mendelian inheritance patterns. Conrad Waddington introduced the term “epigenetic landscape” to describe “the interactions of genes with their environment, which bring the phenotype into being”[1]. Epigenetics is the study of reversible, heritable changes in gene expression that do not involve changes to the underlying DNA sequence—a change in phenotype without a change in genotype. Epigenetic mechanisms are mediated by either chemical modifications of the DNA itself or

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by modifications of proteins such as chromatin that are closely associated with DNA. Some of the best characterized epigenetic modifications thought to initiate and sustain epigenetic changes include DNA methylation, chromatin remodeling, histone modification, and non-coding RNA (ncRNA)-associated mechanisms[2]. Earlier studies showed that heterochromatin and euchromatin are associated with distinct DNA methylation and histone modification patterns that correlate with particular states of gene activity, leading to the concept of an 'epigenetic code' that determines the chromatin state and, consequently, gene expression[3]. In mammals, epigenetic regulation is crucial for a variety of different processes such as development, cell differentiation, and proliferation[4]. A thorough understanding of the regulatory networks and epigenetic mechanisms that underlie context-specific gene expression programs and cellular phenotypes remains a critical scientific goal with broad implications for human health.

Fundamentally, epigenomics, as the name suggests, is the study of the effects of chromatin structure—including higher order chromatin folding and attachment to the nuclear matrix, packaging of DNA around nucleosomes, covalent modifications of histone tails (acetylation, methylation, phosphorylation, ubiquitination), and DNA methylation—on the genetic material of a cell, known as the epigenome. This rapidly expanding field of study is analogous to genomics and proteomics, the study of the genome and proteome of a cell, respectively. The epigenome can differ from cell type to cell type, and in each individual cell it can potentially modulate gene expression in a number of ways—by organizing the nuclear architecture of the chromosomes, inhibiting or facilitating transcription factor access to DNA, and mediating gene expression. Some have referred to these multifaceted aspects of the epigenome as representing a second dimension of the genomic sequence that is pivotal for maintaining cell-type-specific gene expression patterns[5]. The logic behind performing epigenetic analysis on a global level is that inferences can then be made about epigenetic modifications which may not otherwise be possible through analysis of specific loci. The term "epigenomics" differs from "epigenetics" in that the former does not necessarily imply gene memory; in practice epigenomic is used to describe comprehensive analyses of chromatin constituents or of gene regulation. Epigenetics, on the other hand, is often thought to encompass three major types of memory that utilize related mechanisms over different time scales[6]: 1) mitotically heritable transcriptional states established during development (cellular memory), 2) mitotically heritable changes in the responsiveness of organisms to environmental stimuli due to previous experiences where genes can experience a more robust secondary transcriptional response, and 3) meiotically heritable changes in gene expression and physiology of organisms in response to experiences in the previous generations—i.e., where parental experience impacts behavior of the offspring.

The arrival of the first practical massively parallel 'next generation' sequencing (NGS) platform in the mid-2000's marked the beginning of a revolution in genomic research[7,8]. The ability to sequence vast quantities of DNA enables entire genomes or specific targeted genomic regions from many samples to be sequenced accurately and at high depth, which has led to the continued development and refinement of a wide range of applications[8]. The epigenetics community was among the first to capitalize on this development, combining NGS with established methods to capture epigenetically modified genomic regions[9]. A key advantage of NGS platforms is their ability to provide a comprehensive and unbiased view

of the epigenome, freeing investigators from content-limited microarray platforms[9,10]. As in the other genomics fields, epigenomics relies heavily on bioinformatics. Central to being able to unravel the mechanisms underlying the workings of the epigenome at the molecular level is access to robust, reproducible, and streamlined technologies that generate data that can be immediately integrated into existing –omic databases. The goal is to create an ultimate picture of the epigenome integrating DNA methylation, chromatin dynamics and accessibility, and expression. Some of the more established technology platforms of DNA methylation, chromatin profiling, and expression profiling have been extensively reviewed elsewhere[5]. Here we discuss a few of the more recent cutting edge -omic technologic developments created to facilitate the molecular biologists' ability to better interrogate the epigenome, and describe some illustrative examples where application of these methods have provided novel and unexpected insights into the molecular mechanisms of phenotypic plasticity in development and disease.

Higher-order chromosomal structure profiling platforms

It has long been hypothesized that communication between widely spaced genomic elements is facilitated through the spatial organization of chromosomes that brings genes and their regulatory elements together in close proximity[11–13]. Eukaryotic genomes are tightly folded and packaged in a highly organized manner to accommodate the spatial constraints of the nucleus while allowing regulatory factors access to the underlying sequences to affect transcriptional control. The dynamic "folding" of chromatin and chromosome architecture regulates patterns of cellular gene expression during differentiation and development, or in response to environmental signals. Understanding how chromosomes fold can provide insight into the complex relationships between chromatin structure, gene activity, and the functional state of the cell[11,14].

Over the last two decades, numerous studies have assessed the spatial proximity and nuclear organization of specific genomic loci, initially through microscopy techniques such as fluorescent in situ hybridization (FISH), and more recently by chromosome conformation capture (3C[15]). These methods have provided strong evidence that long-range chromosomal interactions are widespread, suggesting a high level of communication between dispersed elements in the genome[16,17]. Specifically, 3C is a molecular technique that uses formaldehyde cross-linking and locus-specific PCR to detect physical contacts between genomic loci[15]. Significant effort over the past few years has focused on obtaining comprehensive mapping of chromosomal interactions. Several adaptations of 3C have been developed that allow large-scale detection of genomic interactions by using microarrays or high-throughput sequencing technologies. The 4C method (3C-on chip, or circular 3C) allows identification of regions throughout the genome that are physically close to a single locus of interest; similarly, 5C (3C–carbon copy) is not anchored on a single locus and is used for mapping dense interaction networks throughout large chromosomal regions of interest. Hi-C, first introduced in 2009, brings these analyses to the -omic level by enabling probing of three three-dimensional architecture of whole genomes through coupling proximity-based ligation with massively parallel sequencing[18]. An increasing number of studies leveraging the power of Hi-C has provided new insight into the global organization of the genome. For example, chromosomes are now believed to be partitioned into

megabase-scale topologically associating domains (TADs[19–21]) and smaller, nested subTADs[22,23]. Conceptually, the TADs/subTADs are thought to represent particular areas of the genome where all pairs of loci interact more frequently with one another than their surrounding regions. The TADs/subTADs can also form higher order "A" and "B" compartments of active and inactive chromatin, respectively[18,23]. Significantly, at each level in the chromatin folding hierarchy, the folding patterns exhibit a complex connection to genome function and dysfunction in models of normal development and disease[24]. The advent of Hi-C has established a burgeoning field for studying chromatin interactomes and regulation networks in 3D, and has been transformative in our ability to understand the architecture of the genome at high resolution.

Chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) is a variation of Hi-C that features an immunoprecipitation step to map long-range DNA interactions[25], producing a directed view of long-range contacts associated with a protein factor of interest. In this method, DNA-protein complexes are crosslinked and fragmented. Specific antibodies are then used to immunoprecipitate proteins of interest, while specific linkers are ligated to the DNA fragments, which ligate when in proximity. Subsequent deep sequencing provides base-pair resolution of ligated fragments, providing a genome-wide unbiased and *de novo* discovery of long-range chromatin interactions. Hi-C and ChIA-PET provide a nice balance of resolution and reasonable coverage in the eukaryotic genome to map long-range interactions.

Genomic analyses of protein-directed chromosomal architecture

Variations on the Hi-C theme have been made in an attempt to achieve enhanced specificity in mapping the relationship between protein binding and the 3-dimensional genome; however, because Hi-C interrogates all possible proximity ligations genome-wide, deep sequencing is required to fully identify chromatin architectural features. Enrichment strategies have been developed to target factor-directed interactions via ChIA-PET (described above) and locus-specific interactions via Capture-C and related methods[26]. One such technique is HiChIP, an rapid, efficient, and technically simplified method for mapping factor-directed chromatin conformation[27] Long-range DNA contacts are first established *in situ* in the nucleus; chromatin immunoprecipitation (ChIP) is then performed on the contact library, directly capturing long-range interactions associated with a protein of interest. High throughput sequencing then identifies two distantly located segments of the genome from one fragment, indicating that the factor of interest was associated with the long-range interaction. An advantage of HiChIP is the dramatically lower cell number necessary to produce high-confidence contact maps[27], which will facilitate future studies of chromatin conformation in systems previously unmeasurable by conventional strategies.

A genomic window into chromatin accessibility

Major insights into the epigenetic information encoded within chromatin have come from high-throughput, genome-wide methods for assaying chromatin accessibility (so called "open chromatin"[28]), nucleosome positioning[29], and transcription factor (TF) occupancy[30]. The drawback with existing methods is that they all require millions (often hundreds of

millions) of cells as starting material, involve complex and time-consuming sample preparation protocols, and cannot easily interrogate the interplay of chromatin accessibility, nucleosome positioning, and TF binding simultaneously in the same sample. Traditionally, enzymes such as micrococcal nuclease and DNase I have been used to preferentially cleave nucleosome-depleted DNA sequences to measure chromatin sensitivity[31] to identify active regulatory sequences in the genome. Genome-wide tools have been developed leveraging the activity of DNase I[32,33] and a prokaryotic transposase enzyme, Tn5, that preferentially integrates into active, open chromatin elements *in vivo*[34]. ATAC-seq (assay for transposase-accessible chromatin using sequencing) takes advantage of hyperactive Tn5 transposase with loaded *in vitro* adaptors for high-throughput DNA sequencing to provide comparable information about unfixed eukaryotic accessible chromatin to that given by the new DNase-seq methods[34]. What sets ATAC-seq apart from the other molecular tools is its simple protocol and low cell number requirement[34], opening up the possibility of assessing chromatin accessibility in samples for which large numbers of cells is not feasible.

Modulation of chromosomal architecture at will

While work over the last two decades have demonstrated a correlation between chromatin topology and the underlying cellular gene activity, the critical question of whether dynamic changes in chromosome folding is a cause or consequence of genome function is still unresolved[35,36]. It would appear from the wealth of accumulated whole genome 3-dimensonal data that chromosomal architecture is a dynamic yet highly organized structure, presumably built progressively from stabilization of functional contacts between genes and their respective regulatory elements. However, direct hypothesis testing regarding how chromatin loops are organized and what their function(s) are has been difficult due to the fact that most of the high dimensional data has been obtained at a population-average level, and that disruption of TAD insulation does not appear to impact higher-order genomic compartmentalization[37]. What has been missing in the field was a heterologous reagent that can be readily programmed to connect any two endogenous DNA segments to facilitate DNA loop engineering, and to molecularly dissect the elements contributing to chromosomal structural borders and/or key architectural loops.

A major advance that begins to address the causal link between chromatin structure and function came with the development of CLOuD9 (chromatin loop reorganization using CRISPR–dCas9), a method to reversibly establish new chromatin loops[38]. Taking advantage of the powerful genome editing technology Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), CLOuD9 uses orthogonal Cas9 species fused to a reversible dimerization domain to target and subsequently bring together any two chromosomal loci in the genome to affect gene expression. An appropriate analogy is that of protein structure—once the structure for a particular protein is resolved, the key residues contributing to the protein's function can be readily identified and engineered; similarly, as knowledge of the formation and maintenance of specific chromatin loops increases, comparable structure-informed reverse genetic engineering will allow one to manipulate the genome, with myriad applications. Similar reagents[39] are being developed that will allow creation of de novo DNA looping in a variety of cell types, aiding understanding of endogenous loops and enabling creation of new regulatory connections. More importantly, mechanistic links can

now be assigned to what were previously only correlations between chromatin conformations and transcriptional regulation (Figure 1A).

Epigenetic editing with CRISPR

Several effective and precise tools have been developed that enable site-specific manipulation of DNA methylation[40–43], directly addressing the relationship between DNA methylation and gene expression while bringing to light a novel approach that can selectively and heritably alter gene expression. The technologies all take advantage of deactivated Cas9 (dCas9) nuclease fused to either the catalytic domain of the DNA methyltransferase (DNMT3A) or Ten-Eleven Translocation methylcytosine dioxygenase1 (TET1), and demonstrate specific DNA methylation activity for the targeted region and heritable effects through mitotic divisions. Together, these tools should allow more mechanistic studies of DNA methylation and its role in guiding molecular processes that determine cellular fate.

From bulk populations to single-cell epigenomics

One of the most exciting and powerful recent developments in epigenomics is the application of technologies allowing analyses at the single-cell level[44,45]. Because epigenetic information is encoded in multiple forms, ranging from covalent modifications on DNA, chromatin accessibility and compaction, post-translational modifications of histones, and higher-order chromosomal conformation, each layer of epigenetic information requires a separate molecular approach to profile it. For many biological questions, observations of epigenetic regulatory systems at the single-cell level will likely elucidate intercellular differences that will lead to a better understanding of the underlying mechanisms compared with bulk analysis[46]. For example, advances in whole-genome and whole-transcriptome amplification have permitted the sequencing of the minute amounts of DNA and RNA present in single cells, offering a window into the extent and nature of genomic and transcriptomic heterogeneity found in both normal development and disease[47]. For instance, a recent single-cell transcriptional profiles of the murine non-myocyte cardiac cellular landscape using single-cell RNA sequencing (scRNA-seq[48]). Detailed molecular analyses of the scRNA-seq data revealed the diversity of the cardiac cellulome and facilitated development of techniques to isolate understudied cardiac cell populations, such as mural cells and glia, offering insights into the structure and function of the mammalian cardiac cellulome and providing an important resource in cardiac cell biology. Indeed, single-cell approaches stand poised to revolutionize our capacity to understand the range and magnitude of epigenomic diversity that occur during the lifetime of an individual organism. In addition, combined single-cell methods are also rapidly emerging that allow analyses of epigenetic–transcriptional correlations at different time scales, thereby enabling detailed investigations of how epigenetic states are associated with phenotype and allowing for discovery of new layers of molecular connectivity between the genome and its functional output[44,45].

Methods to interrogate 3-dimensional chromosomal structure and chromatin accessibility in individual cells have been developed over the last few years, such that these features are now assayable at the single-cell level[44,45]. As discussed above, Hi-C enables measurement of the

proximity of genomic loci in 3-dimensional space. Variations and optimizations have been performed to increase throughput and resolution to the single cell level, a method referred to as single-cell Hi-C (scHi-C[49–52]). Single-cell Hi-C has allowed visualization and reconstruction of the 3-dimensional organization of every chromosome in individual haploid cells[53] and revealed how data from population Hi-C can obscure the dynamic reorganization of chromosome compartments during the cell cycle[51]. The resolution of scHi-C methods is expected to continue to improve such that eventually it will be possible to accurately map contacts between specific promoters and their enhancers. Nonetheless, scHi-C has bridged current gaps between genomics and microscopy analyses of chromosomes, demonstrating how modular organization underlies dynamic chromosome structure, and linking these structures with genome activity patterns[50,51].

Similarly, the resolution of ATAC-seq has been improved with the development of single cell ATAC-seq (scATAC-seq[54]). Taking advantage of microfluidics to process single cells while introducing cell-identifying barcodes as part of the tagging process, scATAC-seq is a robust method that allows parallel processing of a large number of samples to reveal the landscape and principles of mammalian DNA regulatory variation. In addition to providing insights into cell-to-cell variation, scATAC-seq allows identification of specific *trans*-factor and *cis*-elements associated with the variance in cell-type-specific accessibility. Interestingly, scATAC-seq elucidates the pattern of accessibility variation *in cis* across the genome that recapitulates chromosome compartments *de novo*, thereby linking single-cell accessibility variation to 3-dimensional genome organization[54] (Figure 1B).

These single cell epigenomic approaches will ultimately allow a full understanding of genome regulation that involves integrating three different layers of data: one-dimensional data regarding the state of local chromatin (such as patterns of protein binding along chromosomes and the accessibility of chromatin), three-dimensional data describing the population-averaged folding of chromatin inside cells, and single-cell observations of three-dimensional spatial co-localization of genetic loci and trans factors that reveal information about their dynamics and frequency of co-localization. However, despite these advances, there remain significant challenges and limitations that apply to these single-cell epigenome methods. Currently, important bottlenecks include the limited capture rate, low mappability rates, and high levels of PCR duplicates[45]. Improved computational tools will also be required to process, integrate, and visualize connections between the different molecular layers within and between cells.

Applications of epigenomics tools to disease

The advent of the epigenomic tools over the past few years has brought forth a more holistic view of the interplay between the genome and a very active epigenome, forming an causal link between the underlying genome, the regulatory epigenome, and the functional consequences stemming from perturbations in both. Importantly, these tools are being leveraged to uncover mechanisms of complex diseases. Such integrative approaches may provide insights into the causal regulatory mechanisms of disease for purposes of early-stage detection as well as therapeutic development.

One such example is coronary artery disease (CAD). Meta-analyses of genome-wide association studies in humans have identified hundreds of loci associated with CAD and myocardial infarction susceptibility[55,56]. However, the mechanisms and functions of many of these loci have remained unclear due to the fact that a large number of the variants reside in non-coding regions. Quertermous and colleagues applied an integrative approach to investigate some of these causal regulatory variants in CAD using genomic, epigenomic, and transcriptomic analyses with targeted experimental follow-up at selected candidate loci[57]. The authors hypothesized that understanding the epigenetic gene regulatory mechanisms in primary cultured human coronary artery smooth muscle cells (HCASMCs) will provide greater insights into these disease variants as well as the underlying biology of the vessel wall. To begin to dissect the epigenomic changes in CAD, they performed ATAC-seq on stimulated HCASMCs, normal, and atherosclerotic human coronary artery tissue, to generate chromatin accessibility profiles. These were then integrated with ChIP-seq data to define HCASMC-enriched cis-regulatory regions. Publicly available annotations were incorporated to identify several representative loci, and expression quantitative trait loci (eQTL) analyses were done on cohorts of normal and atherosclerotic arteries to validate the endogenous functions of these variants in the appropriate disease environment. Overall, 64 candidate regulatory variants in stimulated HCASMCs and 26 in coronary arteries *ex vivo* were identified; of these candidate variants, the functionality of seven were confirmed via allele-specific binding, enhancer traps and allelic expression imbalance[57].

lncRNAs in cardiac development and disease

Long noncoding RNAs (lncRNAs) are among the several families of noncoding RNAs that have emerged as powerful regulators of cellular and tissue function[58,59]. Initially considered the “dark matter of the genome,” lncRNAs are now regarded as critical epigenetic regulators of gene expression[60]. The regulation of cardiac pathways by lncRNAs is still poorly understood. Numerous studies have revealed that lncRNAs have important roles in healthy and diseased hearts by transcriptome profiling in cardiac tissues and identifying hundreds of differentially expressed lncRNAs. Several integrative approaches have linked lncRNAs with specific biological functions, such as modulating chromatin states, regulating transition of chromatin state during cardiomyocyte differentiation, and affecting physiological traits implicated in cardiac remodeling[61]. Other lncRNAs, such as MHRT[62], dictate cardiac chromatin signatures through binding of the chromatin repressor complex. Other lncRNA functions include regulation of cardiomyocyte metabolism, hypertrophy, differentiation, and proliferation[63]. Whether lncRNAs participate in the regulation of inflammation and fibrosis, both of which represent hallmarks of cardiac remodeling, remains to be determined. Of course, not all differentially expressed lncRNAs will turn out to be functionally important, but their unique association with chromatin states and enhancers suggests fundamental signaling roles. Due to space limitations we refer interested readers to recent reviews on lncRNAs[64,65] and microRNAs[66,67] in cardiovascular research, as both have been extensively studied as mechanistic regulators of cardiovascular development and disease and potential therapeutic targets.

Elucidating the principles governing the enhancer connectome to identify targets of disease-associated DNA elements

The inability to casually link intergenic mutations to their presumed target genes has limited a better molecular understanding of human diseases. This is a particularly glaring gap as the majority of inherited risk factors for common diseases reside in intergenic enhancers and noncoding features in DNA[68]. Taking advantage of the HiChIP technology[27], Mumbach and colleagues set out to define the high-resolution landscape of enhancer–promoter regulation in primary human cells. They generated high-resolution contact maps of active enhancers and target genes in primary human T cell subtypes and coronary artery smooth muscle cells through a series of H3K27ac HiChIP experiments[69]. Perhaps not surprisingly, enhancer–promoter contacts were found to be highly dynamic in related cell types and to often involve genomic elements with shared accessibility. Functions were assigned to autoimmune and cardiovascular disease risk variants from genome-wide association study (GWAS)-identified SNPs; the SNPs were also subsequently linked to their putative target genes (Figure 2). These target genes were further validated through modulation of the linked enhancers by CRISPR gain and loss of function assays, and through correlation with expression quantitative trait loci and allele-specific enhancer loops in primary cells from patients. Importantly, because HiChIP demonstrated that the majority of disease-associated enhancers contact their targets beyond the nearest gene(s) in the linear genome, these data also expand the number of potential target genes for autoimmune and cardiovascular diseases. Moreover, these results solidify the idea that mapping chromosomal structural conformation in primary cells can lead to the identification of novel, often hidden, regulatory connections underlying gene function in human disease.

Hi-C applications to disease

Mounting evidence suggests that the unique genome configurations of loops and chromosome territories may be linked to the establishment and/or maintenance of human disease phenotypes[70–72]. For instance, the spatial proximity of chromosomes directly influences the probability of translocation between specific loci[73]. In fact, 3D domain disruption of TAD and sub-TAD boundaries via mutations, deletions, or genetic rearrangements has been linked to aberrant activation of genes via ectopic looping of enhancers in limb malformation syndromes[70] and cancer[71]. Mutations affecting proper function of architectural proteins such as cohesin and the structural properties of the nuclear lamina give rise to diseases known as cohesinopathies[74] and laminopathies[75], respectively. Additionally, how the genome folds in 3D space facilitates connection of distal SNPs with their target genes was demonstrated when a functional link between a mutation in an intron of the *fat mass and obesity associated* (*FTO*) gene and an evolutionarily conserved aberrant enhancer-promoter contacts to IRX3, a novel determinant of body mass and composition, was established[76]. Recently, Hi-C was used to investigate the mechanisms of epigenomic function in adult cardiac myocytes, using a murine model of pressure overload–induced hypertrophy[77]. Chromatin capture was used to determine the structure of the cardiac myocyte epigenome, providing a high-resolution resource of the endogenous chromatin architecture in cardiac myocytes while specifically delineating the global changes in

chromatin interactions during heart failure. The role of the chromatin structural protein CCCTC-binding factor (CTCF) was also examined by using an *in vivo* loss-of-function model, revealing its potential role in remodeling long-range interactions of cardiac enhancers and changes in larger scale genome accessibility. These findings demonstrate the promise of epigenomic technologies as a novel means of exploring the 3-dimensional features of the cardiac nucleus, and support epigenomic plasticity as a common feature of cardiac pathophysiology induced by distinct stimuli.

Thus, aberrant 3D chromosomal structure represents a new dimension through which an understanding of sporadic and familial disease states can be made, leading to unraveling of novel therapeutic interventions based on preventing or rewiring pathological 3D contacts. Together, these epigenomic studies support an emerging model in which genome folding and misfolding are critically linked to the onset and progression of a broad range of human diseases. Knowledge of the dynamics of genomic interactions in disease may enable new strategies for therapeutic intervention. Novel technologies to manipulate the epigenome and the 3D chromatin, such as the ability to create novel chromatin contacts and loops at will (CLOuD9[38]), should allow heretofore unavailable investigations into the mechanisms and sequelae of both cis- and trans-chromosomal interactions to be performed.

Epigenomics as a conduit to personalized “regulomes”

As a whole, epigenetic variation can yield information on cellular states and developmental histories in ways that genotype information cannot. Furthermore, in contrast to fixed genome sequences, epigenetic patterns are plastic, and regulated gene expression plays key roles in nearly every developmental program and disease state. This type of temporally sensitive and dynamic information feedback between perturbation and outcome is essential to tailor precise medical treatments for individual patients. Manipulating aberrant, disease-causing epigenetic marks would thus appear to hold considerable therapeutic promise.

The rapid development of high-throughput technologies and computational frameworks have allowed researchers to examine biological systems in unprecedented detail. The ability to study biological phenomena at the -omics levels in turn can be expected to lead to significant advances in personalized and precision medicine. Patients can be treated according to their own molecular characteristics. Individual -omes as well as the integrated profiles of multiple –omic information are expected to be valuable for health monitoring, preventative measures, and precision medicine, transforming medical care from traditional symptom-oriented diagnosis and treatment of diseases toward disease prevention and early diagnostics. Furthermore, regulome analysis may directly investigate chromatin or TF pathways that are direct drug targets.

Some groups have already begun to take advantage of these sensitive genomic technologies. For example, ATAC-seq was utilized to better visualize the personal regulome from a standard blood draw, the most common source of human samples for clinical diagnostics[78], where the authors provided foundational data and methods to compare and visualize differences in personal regulomes. The number, location, and potential sources of *in vivo* variation in chromatin accessibility on a genome-wide scale were identified and analyzed,

opening up the possibility that potential variation in chromatin accessibility in the population may be a key to understanding and managing healthy and diseased states. Finally, comparisons of regulome variation in healthy versus diseased patients documented the feasibility of using the personal regulome approach to investigate disease bio-markers and mechanisms[78].

Moreover, the newer single cell technologies have been shown to be compatible with the small sample sizes of human biopsies and clinical workflows. A drawback to current therapeutics is their non-specific effects. Development of locus-specific epigenetic modifiers, used in conjunction with epigenetic biomarkers of response, will enable truly precision interventions. Using these epignomic tools to monitor personal regulomes in health and disease offers many exciting possibilities.

Conclusion

What more needs to be done to understand the complete epigenome? For the most part, we are still collecting in the initial phases of discovering the components. Just as the full sequence of a genome has greatly facilitated progress in genetics, a clearer understanding for epigenetics will likely come when all the parts are known. It is encouraging to see the great strides that have been made in the last decade, and the anticipated advances and challenges in systems biology-powered personalized medicine that will define the future of personalized health care that is now becoming unraveled.

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Nonstandard Abbreviations and Acronyms

FISH	fluorescent in situ hybridization
NGS	next generation’ sequencing
3C	chromosome conformation capture
TADs	topologically associating domains
ChIA-PET	Chromatin interaction analysis by paired-end tag sequencing
CLOuD9	chromatin loop reorganization using CRISPR–dCas9

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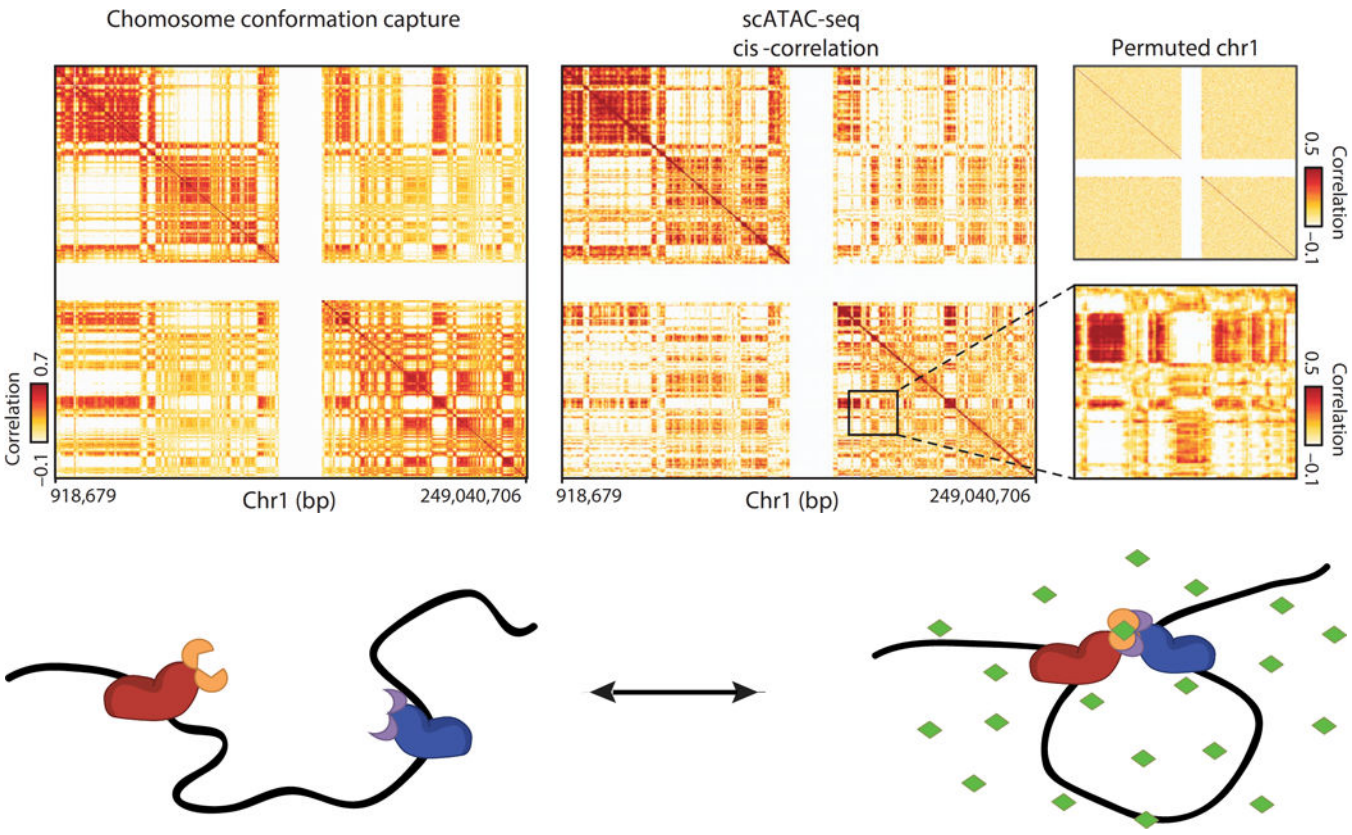

Figure 1.

(A) **Measurement of long-range contact of DNA elements highlighted by single-cell ATAC-seq (scATACT-seq)**. Structured cis-variability across single epigenomes highlighted by single-cell ATAC-seq (scATACT-seq). Pearson correlation coefficient representing chromosome compartment signal of interaction frequency from a population chromatin conformation capture assay (left panel) or scATAC-seq (middle panel) from chromosome 1. Data in white represents masked regions due to highly repetitive regions. (right panel, upper box) Permuted cis-correlation map for chromosome 1. (right panel, lower box) Representative region depicting long-range covariability. From Buenrostro et al., 2015.

(B) **Schematic of CLOuD9 as a reversible method for manipulating chromosomal loops**. Addition of abscisic acid (ABA, green) brings two complementary CLOuD9 constructs (CLOuD9 *S. pyogenes* (CSP), CLOuD9 *S. aureus* (CSA), red and blue, respectively) into proximity, remodeling chromatin structure. Removal of ABA restores the endogenous chromatin conformation. From Morgan et al., 2017.

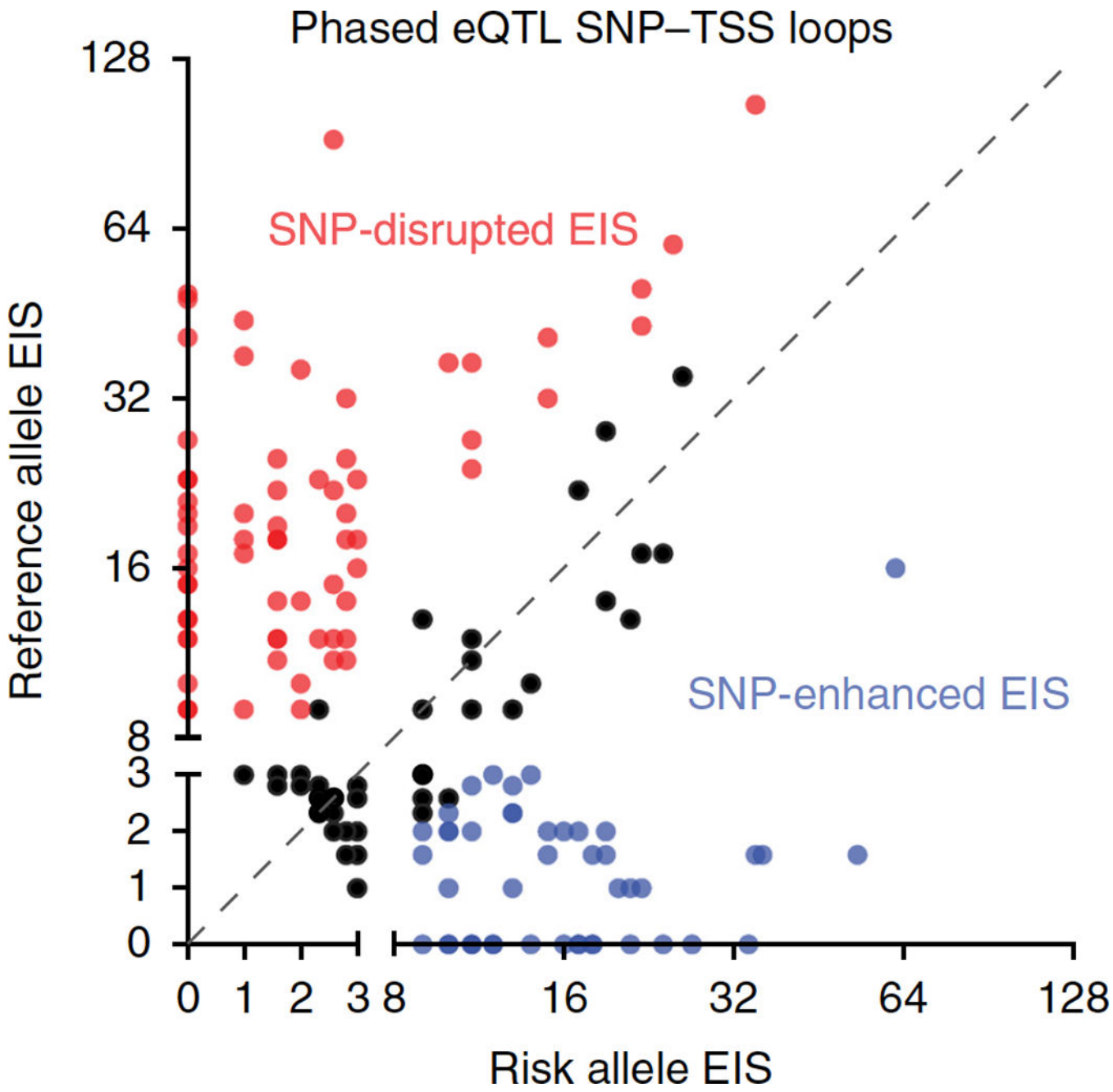

Figure 2. HiChIP identifies allele-specific loops in coronary smooth muscle of coronary artery disease-associated single nucleotide polymorphisms

Genome phasing information in human coronary artery smooth muscle cells (HCASMCs) was used to measure enhancer–promoter interactions at allele-specific CAD-associated SNPs, allowing the examination of functional consequences of risk variants compared to their alternative alleles for a set of CAD-associated SNP–target genes. Many risk alleles disrupted enhancer–target gene interactions (red), but a subset of pathogenic SNPs increased enhancer–target gene interactions (blue). From Mumbach et al., 2017.