OPINION

Making the case for chromatin profiling: a new tool to investigate the immune-regulatory landscape

Deborah R. Winter, Steffen Jung and Ido Amit

Abstract | Recent technological advances have enabled researchers to accurately and efficiently assay the chromatin dynamics of scarce cell populations. In this Opinion article, we advocate the application of these technologies to central questions in immunology. Unlike changes to other molecular structures in the cell, chromatin features can reveal the past (developmental history), present (current activity) and future (potential response to challenges) of a given immune cell type; chromatin profiling is therefore an important new tool for studying the immune-regulatory networks of health and disease.

All haematopoietic cells in the human immune system have identical genomes and, yet, each cell type has a unique role in keeping the body healthy. Since the completion of the Human Genome Project in 2001 (REFS 1,2), it has become clear that there is considerably more to understanding the human blueprint than simply sequencing the genome. This realization spawned many global studies that aim to uncover the underlying regulatory networks, which include both *cis*-acting elements encoded in the DNA sequence and *trans*-acting binding factors that together coordinate cell-type specification. The past decade has seen massive progress in the field of genomics, and a new approach to understanding gene regulation has emerged: namely, chromatin profiling.

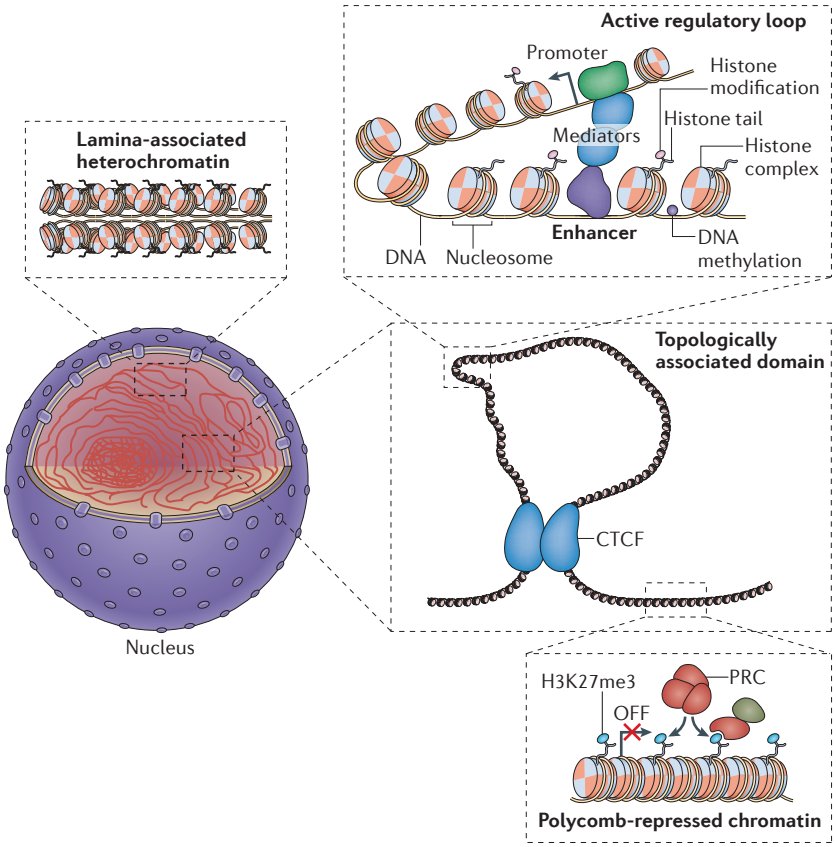
In 2012, the Immunological Genome Project (ImmGen) completed its first phase by profiling the transcriptomes of 249 immune cell types[3–5]. ImmGen has provided the immunology community with a valuable resource, but the collection of transcribed RNAs in a given cell type reveals only one part of the larger regulatory network. With only 1–2% of the human genome spanning protein-coding genes[1,2], the remaining DNA landscape incorporates an abundance of *cis*-acting regulatory elements that contribute to the establishment and maintenance of cell type identity. Analysing RNA expression levels provides only a partial view of the cell's terminal identity without giving a thorough indication of the underlying mechanisms. To learn more about the purpose of the genome beyond the gene sequence, we turn to what lies literally 'over the genome', the epigenome. In particular, this includes the structure and organization of chromatin within the cell's nucleus (BOX 1). Each cell type has its own unique chromatin state, which reflects the regulatory network that defines the specific identity and function of that cell. The location and function of *cis*-acting regulatory elements can be revealed through chromatin profiling assays. In fact, chromatin state is an important regulatory determinant for lineage specification, cellular response to environmental stimuli and defining cell roles within the immune system. Whereas mRNA expression profiling provides a snapshot of the current state of a cell, understanding the epigenomic regulation can give perspective on how this state was reached and how cells might advance. With the common use of high-throughput sequencing, many chromatin assays have now been adapted for genome-wide application. Thus, one can determine the specific catalogue of regulatory elements within a particular cell type that develop, react and interact with each other to modulate gene expression over space and time. Indeed, chromatin profiling offers enormous potential to increase our knowledge of the regulatory landscape in the immune system in both health and disease.

Haematopoietic cells were among the first cell types for which the genome-wide organization and structure of chromatin were profiled in humans, and these pioneering studies established the basic principles of epigenomics that have since defined the field[6–8]. In turn, chromatin profiling has provided insights into several key areas that are of the foremost interest in immunology research by addressing how crucial immune genes are controlled in a cell-type-specific and tissue-specific manner in both health and disease. For example, studies of resting and lipopolysaccharide (LPS)-stimulated *in vitro*-cultured macrophages have shown that the majority of enhancers regulating innate immune response genes are pre-bound by the transcription factor PU.1 and are, consequently, marked by a poised chromatin state before stimulation. By contrast, a few latent enhancers are activated *de novo* upon stimulation of the cell but retain a poised state after the stimulus is removed, thereby functioning as a form of cell memory in anticipation of future restimulation[9,10]. By examining the chromatin state of genetic variants at the *BCL11A* (B cell lymphoma 11A) locus in humans, an intronic enhancer required for erythroid-cell-specific expression was found to influence fetal haemoglobin levels[11]. In a follow-up experiment using a mouse model, the authors confirmed that mutating this enhancer de-repressed embryonic haemoglobin expression in erythroid cells without influencing other cell types, suggesting a possible treatment for sickle cell anaemia. Similarly, cross-referencing the genome-wide association study (GWAS) database of disease-causing variants with genome-wide chromatin profiles in specific immune cells has highlighted the role of *IKZF3* — which encodes an IKAROS family transcription factor involved in lymphocyte differentiation — in multiple sclerosis[12]. These and many other studies demonstrate the potential of chromatin profiling in guiding the development of new therapies and extending our basic understanding of how haematopoiesis and immunity are encoded in our genomes.

In this Opinion article, we describe, with examples, how chromatin profiling can be used to make novel discoveries and reinforce classical immunological studies. We highlight the recent advances in experimental technologies that have made chromatin profiling more practical and efficient for use in everyday immunological research. We discuss the approaches that have developed from early chromatin studies in mouse cells and cell culture models to gain insight into the past, present, future and environmental response of immune cell types. In many cases, the global catalogue of regulatory elements in the genome that are elucidated by chromatin profiling can also be used to infer the activity and binding of transcription factors. We propose a path for translating chromatin studies into humans, taking into consideration the issue of genetic variation, which constitutes an added hurdle for clinical applications but also has the potential of unique rewards for direct translation to disease therapy. We conclude with our vision of the future of immunological research, including the regular use of chromatin profiling and public epigenomic databases in studies of the immune-regulatory landscape. We direct readers to the specific references throughout the text for exact methodological protocols and analysis, as well as to details of the current endeavours by consortia to systematically map the epigenome (see Further information); our aim here is to give the reader a meaningful overview and impression of the potential of this approach. Although we cannot cover all of the noteworthy studies that have profiled chromatin in immune cells, we hope to convey the extensive benefits of this approach, the challenges and the exciting possibilities ahead.

Box 1 | **Chromatin**

The DNA in the nucleus of all eukaryotic cells is organized into chromatin (see the figure). The basic unit of chromatin is the nucleosome, which consists of approximately 147 base pairs of DNA wrapped approximately 1.7 times around a histone protein complex[69]. The histone complex consists of two copies each of H2A, H2B, H3 and H4. Nucleosomes may change position, which alters the accessibility of the underlying DNA, or they can be post-translationally modified by proteins known as chromatin remodellers[70]. These histone modifications are designated according to their location in the histone tail and the type of modification (such as methylation, acetylation, phosphorylation or ubiquitylation)[71]: for example, H3K4me2 indicates that the H3 tail is dimethylated on the fourth lysine. Regions of the genome that are nucleosome depleted, known as 'open chromatin', are enriched for functional regulatory elements, are accessible to transcription factor binding and typically coincide with active histone modifications[17,72]. Promoters, which are marked by H3K4me3, tend to be constitutive (found in all cell types); by contrast, enhancers, which are marked by H3K4me1, form a more cell-type-specific regulatory landscape. Tightly bound chromatin, known as heterochromatin, often contains stably repressed, inaccessible genomic elements and is situated closer to the nuclear perimeter or lamina[73,74]. On a higher level, chromatin is arranged within the nucleus to form a 3D architecture through a process that is mediated by structural proteins. For example, two copies of CTCF bound at adjacent chromatin boundaries will come into contact with each other, as well as cohesin and other mediators . In this way, interacting elements (such as enhancers) that are separated by a genomic distance can be brought into physical contact within DNA loops. Studies have shown that similarly regulated regions form large compartments including topologically associated domains (TADs) of active regulatory elements and tracts silenced by the polycomb-repressive complex (PRC)[75,76]. Each cell type has a distinct global chromatin state that reflects the activity of its regulatory landscape.



Advances in chromatin technology

Profiling the complete chromatin landscape of all cells in the haematopoietic system is a formidable task. Within the main cell lineages, many cell subtypes have been defined and each one of these must be individually isolated and analysed. Taking into account external influences and the sensitivity of cells to the local environment, distinct cell populations — such as those in different tissues — should be analysed separately[13,14]. Furthermore, primary cells should be analysed immediately after isolation, as growth in culture following removal from their natural context is likely to influence the underlying chromatin regulatory landscapes. This was recently demonstrated for tissue-resident macrophages, which rapidly lost their tissue-specific chromatin signatures upon isolation and culture[13].

The general approach of chromatin studies is to isolate and enrich for genome fragments that are associated with the chromatin feature of interest (for example, nucleosomes, open chromatin, DNA methylation or histone modifications) to create libraries for sequencing (FIG. 1). The challenge of traditional genome-wide studies is that due to the low efficiency of certain stages of the protocol, a large starting population of cells is needed to produce the large number of unique sequences that are required to adequately sample all regulatory elements. In this way, the entire epigenomic network can be constructed *de novo* without

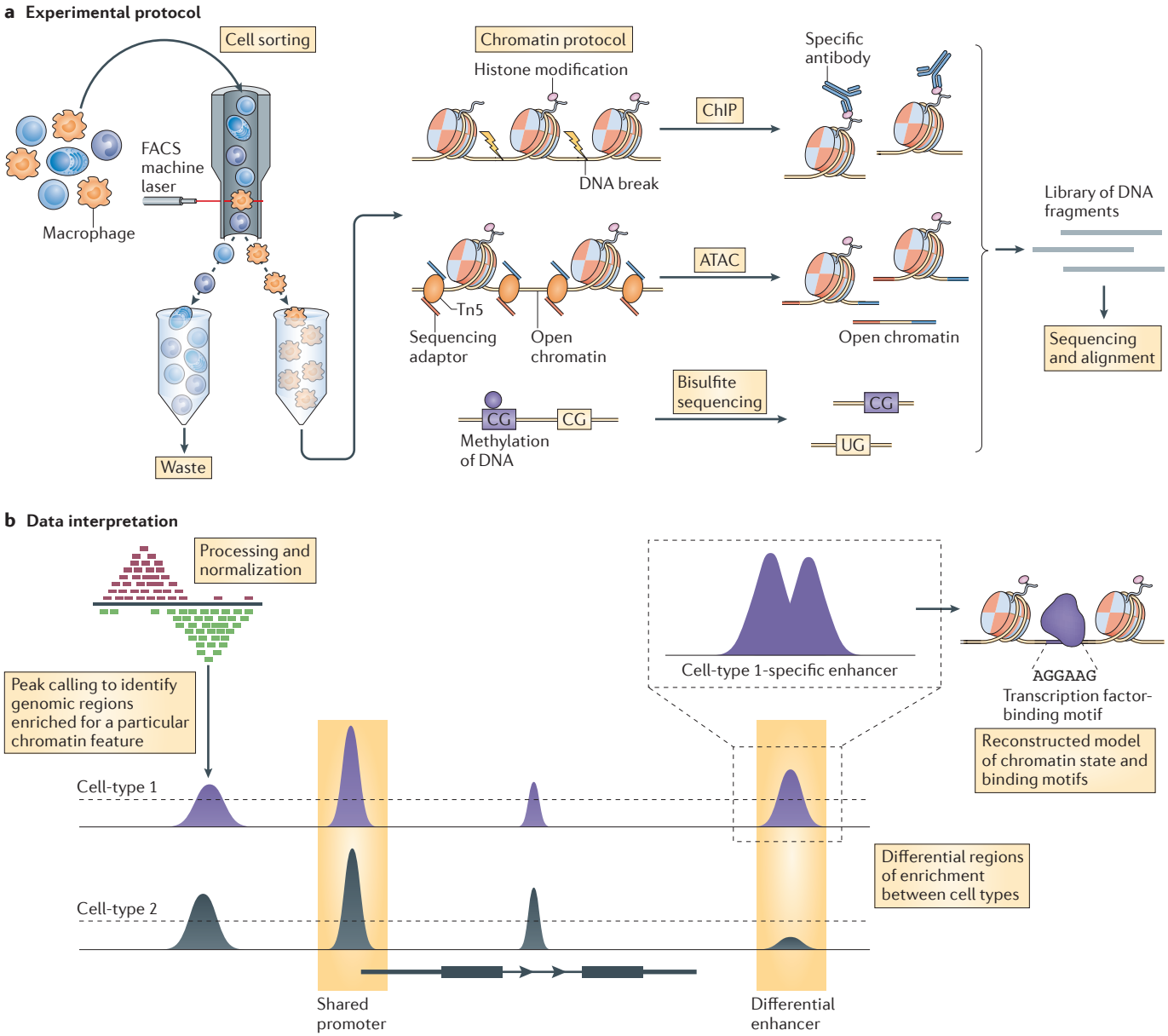


Figure 1 | **Representative chromatin profiling: from immune cells to the regulatory landscape.** **a** | Experimental protocol. In this example, the cell type of interest (macrophages) is isolated from other cells by fluorescence-activated cell sorting (FACS). Next, the chosen protocol for chromatin profiling is carried out depending on the type of data desired. The purpose of each protocol is to create a sequencing library enriched for genome sequences that associate with the particular chromatin feature of interest. Shown here are chromatin immunoprecipitation (ChIP), assay for transposase-accessible chromatin (ATAC) and bisulfite sequencing. The ChIP assay uses antibodies to locate a specific histone modification followed by, typically, sonication to create sequencing fragments[6,67,77]. ATAC identifies open chromatin regions through the binding of Tn5 transposase and insertion of sequencing adaptors[16]. Bisulfite sequencing recognizes DNA methylation by converting unmethylated cytosines to uridines that will be recognized during sequencing[19]. The fragments in these libraries are then sequenced and can be aligned to the reference genome using one of several open-access algorithms. **b** | Data interpretation. The next steps are to process and normalize the aligned sequences so that the results can be visualized (for example, in a genome browser). Bioinformatic analysis is carried out to interpret the data on a global level and to reconstruct the original chromatin state including: identifying genomic regions that are enriched for a particular chromatin feature (peak calling); characterizing differential regions of enrichment between cell types; annotating the regulatory elements in these regions; finding transcription factor-binding motifs; and reconstructing the regulatory network. Dashed horizontal lines represent the threshold for peak calling.

prior knowledge of the important genes, and global patterns and key regulatory factors in the cell can be identified. For most immune cell types *in vivo* (particularly precursors and rare subtypes, and for patient samples), obtaining sample sizes sufficient for classical analytical approaches has been exceedingly difficult. Therefore, until recently, it was not possible to carry out high-throughput chromatin assays on these cells.

Fortunately, several common chromatin technologies have recently been successfully optimized to considerably increase both efficiency and sensitivity[15,16]. This was achieved by improving the recovery rate for the

sequences of interest, as well as by decreasing the inherent 'noise' of irrelevant genomic regions. Specifically, the iChIP approach — which enhances the traditional high-throughput chromatin immunoprecipitation (ChIP) assay for profiling histone modifications — uses a double round of antibody enrichment (one general and one specific), as well as pooling of multiple samples, to increase signal and reproducibility. To replace assays for open chromatin that require an input of millions of cells, such as the DNase hypersensitivity assay[17], the assay for transposase-accessible chromatin (ATAC) uses the Tn5 transposase to efficiently fragment and barcode the genome[16,18]. Both iCHIP and ATAC enable practical *in vivo* genome-wide chromatin profiling of less than a thousand sorted immune cells directly[19]. In parallel with these experimental advances, much progress has been made in data interpretation, and better algorithms for alignment, normalizing, visualizing, peak calling, motif finding and modelling the regulatory network are now available. The community, in general, has raised the threshold for the quality of both the raw data and analysis involved. A comprehensive map of the chromatin and regulatory network dynamics for the mammalian immune system is now within reach.

In the next section, we describe various epigenomic approaches and the information that they provide about the cell's regulatory network. The principles of these approaches have been used to probe models for processes as diverse as ageing and immune memory; such pioneering studies provide a glimpse of the enormous potential of chromatin profiling.

Glossary

Assay for transposase-accessible chromatin
(ATAC). A method for identifying regions of open chromatin by using Tn5 transposase to insert paired sequencing adaptors into accessible chromatin.

Chromatin
The 3D complex of DNA and proteins within the nucleus. Features of chromatin (including localization, structure, interacting proteins, accessibility and modifications) regulate cell-type-specific gene expression.

Chromatin immunoprecipitation
(ChIP). A method for identifying genomic regions that associate with a particular protein, including specifically modified histones, through immunoprecipitation of the crosslinked DNA fragments.

Chromatin interaction analysis using paired-end tag sequencing
(ChIA-PET). A method for identifying pairs of regions associated with a particular protein by combining chromatin immunoprecipitation with the isolation of interacting DNA fragments.

***Cis*-acting elements**
Functional regulatory elements (such as enhancers) within the genome that regulate the transcription of genes.

DNA methylation
The addition of a methyl group to a nucleic acid in the genome, typically to cytosine within a CpG pair.

DNase hypersensitivity assay
A method that relies on the preference of the enzyme DNaseI to digest unbound DNA to identify regions of open chromatin.

Enhancers
Distal regulatory elements that may function in combination with promoters or other enhancers to influence the transcription of one or more genes through the binding of transcription factors.

Epigenome
Modifications to the genome that do not change the DNA sequence, including DNA methylation, histone modifications and rearrangements of chromatin structure.

Expression quantitative trait loci
(eQTLs). Genomic loci that correlate with changes in gene expression analogously with the relationship between quantitative trait loci and phenotype.

Formaldehyde-assisted isolation of regulatory elements
(FAIRE). A method for identifying regions of open chromatin by isolating DNA fragments that are not bound to DNA after crosslinking.

Genome-wide association study
(GWAS). A statistical analysis comparing the occurrence of multiple common genetic variants (usually single-nucleotide polymorphisms) with a certain outcome (a phenotype or disease) to identify causal variants.

Hi-C and 5C
High-throughput adaptations of the chromosome conformation capture (3C) method that isolates DNA fragments interacting with each other. 5C searches for interactions between many loci, whereas Hi-C searches for all possible interactions in the genome.

Pioneer transcription factors
Sequence-specific DNA-binding proteins that target closed chromatin sites and recruit chromatin remodellers to transform these sites into an open chromatin state enabling the binding of additional transcription factors.

Promoter
A proximal regulatory element that is typically located upstream of the particular gene it regulates and includes the binding sites of general transcription factors such as RNA polymerase II.

Single-nucleotide polymorphisms
(SNPs). Mutations (substitutions, insertions or deletions) of an individual nucleotide in the genome sequence that are common in the population.

***Trans*-acting binding factors**
Non-DNA molecules (typically referring to transcription factors or chromatin factors) that regulate the transcription of genes.

Inferring a spatiotemporal cell view

Beyond the present activity of the cell, chromatin profiling can provide 'behind-the-scenes' information about how a cell reached its current state and about its ability to adapt to changing circumstances. Different chromatin features inform as to the cell's developmental history, current regulatory network and potential response to challenges, as well as to the impact of the local tissue environment.

The past: developmental history. The past history of a cell consists of numerous small-scale decisions to turn genes on and off in a manner that will in combination lead to its current state. In haematopoiesis, these decisions affect the maintenance of multipotency in haematopoietic stem cells (HSCs) and their differentiation into haematopoietic effector cells of the erythroid, myeloid or lymphoid lineage. Developmental decisions leave a lasting imprint on the chromatin state that subsists over time. DNA methylation, a crucial element of epigenomic regulation throughout development, is associated with gene repression[20]. For example, in HSCs, methylation must be actively maintained to uphold the multipotent state by silencing the expression of cell fate genes that would otherwise reduce proliferation[21,22]. Interestingly, the inhibition of maintenance methylation (through mutation of DNA methyltransferase 1 (DNMT1)) led to reduced lymphoid lineage commitment but had no effect on myeloid commitment, suggesting that methylation is unnecessary for the latter[21]. Mutations in the gene encoding DNMT3A, the methyltransferase responsible for *de novo* methylation, were found to be common in HSCs of patients with acute myeloid leukaemia (AML); the mutated HSCs survived chemotherapy and even had a repopulation advantage over non-mutated HSCs[23]. During the process of differentiation from HSCs into mature immune cells, DNA methylation functions in combination with histone modifications to generate the catalogue of functional and repressed regulatory regions that are necessary for each stage of differentiation[24]. A study measuring genome-wide methylation levels during haematopoiesis identified genes with previously unknown roles in the decision between myeloid and lymphoid lineage commitment — including *Arl4c* (ADP-ribosylation factor-like 4C) and *Jdp2* (JUN dimerization protein 2)[22]. Haematopoietic factors alter the methylation state as the cell matures so that lineage-specific genes are activated and alternative fates are silenced[20]. As a result, each

precursor intermediate in the differentiation process involves a set of methylation and demethylation events that ultimately leads to the mature cell type[22]. A study comparing HSCs from aged and young mice found that widespread shifts in chromatin state — such as decreased methylation of binding sites for transcription factors associated with HSC multipotency (for example, stem cell protein (SCL; also known as TAL1), LIM domain-binding protein 1 (LDB1) and runt-related transcription factor 1 (RUNX1)) — led to the reduced differentiation of HSCs seen in ageing[25]. In the absence of further interference, the methylation status of a cell is preserved through division and thus provides a link to the cell's past.

The present: current regulatory network. Chromatin profiling can also expand our understanding of a cell's present regulatory state. In general, genome-wide measures of chromatin accessibility — such as DNase hypersensitivity assays, formaldehyde-assisted isolation of regulatory elements (FAIRE) and ATAC — which can be combined with high-throughput sequencing (DNASE-seq, FAIRE–seq and ATAC-seq), are useful in identifying which regulatory elements are accessible at a particular time[16,17,26]. Specific histone modifications, as assayed by ChIP–seq, have been useful in classifying diverse regulatory elements[6,27]. Acetylation, particularly histone 3 lysine 27 acetylation (H3K27ac), is further indicative of active enhancer elements and is closely connected with the expression of their associated genes[28]. Indeed, the activity of regulatory elements together with their proximity to transcription start sites may be used to match them to the particular genes that they regulate. A study from the Encyclopedia of DNA Elements (ENCODE) consortium suggested that the genes that are regulated by distal enhancers could be determined by correlating the pattern of chromatin accessibility at an enhancer with that at the gene promoter[17]. However, this approach assumes direct regulation in a linear genome. To take into account combinatorial interactions and the compacted structure of the genome, an alternative approach assays the 3D architecture within the nucleus by chromatin capture experiments, such as Hi-C and 5C, to identify physical interactions between genomic regions[29,30]. These approaches led to the conclusion that not only can a single enhancer regulate multiple genes but also a single gene can be regulated by multiple enhancers (FIG. 2a). Genes encoding proteins with immune functions were among those with the most complex combinations of regulatory elements: the fact that each cell type has many more enhancers than genes suggested that different immune cell lineages may use alternate enhancers to activate the same gene[17]. In fact, a study using chromatin interaction analysis using paired-end tag sequencing (ChIA-PET) technology showed that many mouse genes, including *Myc*, physically interact with an entirely different set of enhancers in B cells than in embryonic stem cells[31]. Moreover, comparison of the *cis*-acting regulatory elements in B cells versus macrophages identified thousands of differential regions bound by PU.1 that are likely to regulate overlapping sets of genes[10]. These regulatory intricacies are examples of how chromatin profiling can reveal more of the cell's active regulatory state than can be discerned from RNA profiling alone.

The future: response to challenge. The future activity of a cell, in terms of both developmental pathways and the response repertoire to stimuli, can also be viewed through chromatin profiling. Many regulatory elements exhibit marks of potential, rather than current, activity. Some of these regions, termed 'bivalent domains', are distinguished by a combination of active (H3K4 trimethylation (H3K4me3)) and repressive (H3K27me3) histone modifications, which are mostly found in promoter regions[6,32,33]. Early on, bivalent domains were identified in embryonic stem cells near genes encoding transcription factors (including SOX, forkhead box (FOX), Iroquois homeobox (IRX), POU and paired box (PAX) family members) and in $CD4^+$ T cells (such as the intergenic region between *RAB6IP2* and *FBXL14*)[6,33]. Bivalent domains are most often observed early in development, foretelling the role of the respective factors later in differentiation. Depending on the cell's eventual differentiation fate, these regions may lose their active or repressive marks to become silenced or activated, respectively. For example, when comparing $CD133^+$ multipotent cells with differentiated $CD36^+$ erythrocyte precursors, about a quarter of the bivalent domains identified in $CD133^+$ cells remain in this state in $CD36^+$ cells, whereas half of the bivalent domains lose only H3K4me3 active marks and most of the remaining regions lose H3K27me3 repressive marks as well as gaining additional activating histone modifications as they differentiate[32]. The combination of these active and repressive histone marks can establish a poised state at genes that define the possible lineages of the cell's progeny.

Alternatively, distal regulatory elements marked by H3K4me1 in the absence of H3K27ac (poised enhancers) can have a similar role in forecasting the activation of lineage-specific genes, and they provide intriguing clues about the complex regulatory dynamics of haematopoiesis[28,34] (FIG. 2b). The ratio of poised to active enhancers in a cell gives a measure of its differentiation potential, with precursors tending to have more poised enhancers than their successors. Comparing cell types throughout the mouse haematopoietic system indicated that 'poising' is a widespread phenomenon, with 32% of the enhancers that are active in differentiated cells having been initially poised in lineage progenitors[15]. Such cases of precise regulation can be crucial for health: *S100a8* —which is marked by H3K4me1 in common myeloid progenitors (CMPs) before the addition of H3K27ac and its expression in neutrophils and other terminal myeloid cells[15] — has an important role in the immune response, and its overexpression leads to chronic inflammation such as that seen in patients with rheumatoid arthritis[35]. The enhancer near *Cx3cr1* (CX_3C-chemokine receptor 1), which encodes a chemokine receptor that is expressed during the development of all macrophages, is poised in CMPs but then reactivated only in intestinal macrophages and microglia[14,15], and it is possibly actively repressed by REV-ERB (also known as NR1D2) in most other tissue-resident macrophages[36]. Within the B cell lineage, poising of enhancers in pro-B cells indicates the future potential of genes such as *Egr3* (early growth response 3) and *Cd40* to be expressed in late B cell stages[37]. Moreover, poised enhancers persisting in mature immune cells may indicate how they will respond to environmental stimuli. In macrophages and dendritic cells, previously poised enhancers recruit signal-dependent transcription factors of the nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) families upon LPS treatment and gain activating histone marks associated with the expression of immediate early genes[9,38]. Poised enhancers, together with *de novo*-activated or latent enhancers, may retain activating marks after the stimulation is removed in preparation for future challenges through a process termed 'trained immunity' (REFS 9,39–41). For example, in bone marrow culture-derived macrophages, the chromatin state was shown to function as a memory of previous immune challenges, enabling genes that continued to be inducible after Toll-like receptor (TLR) stimulation (non-tolerizable), including

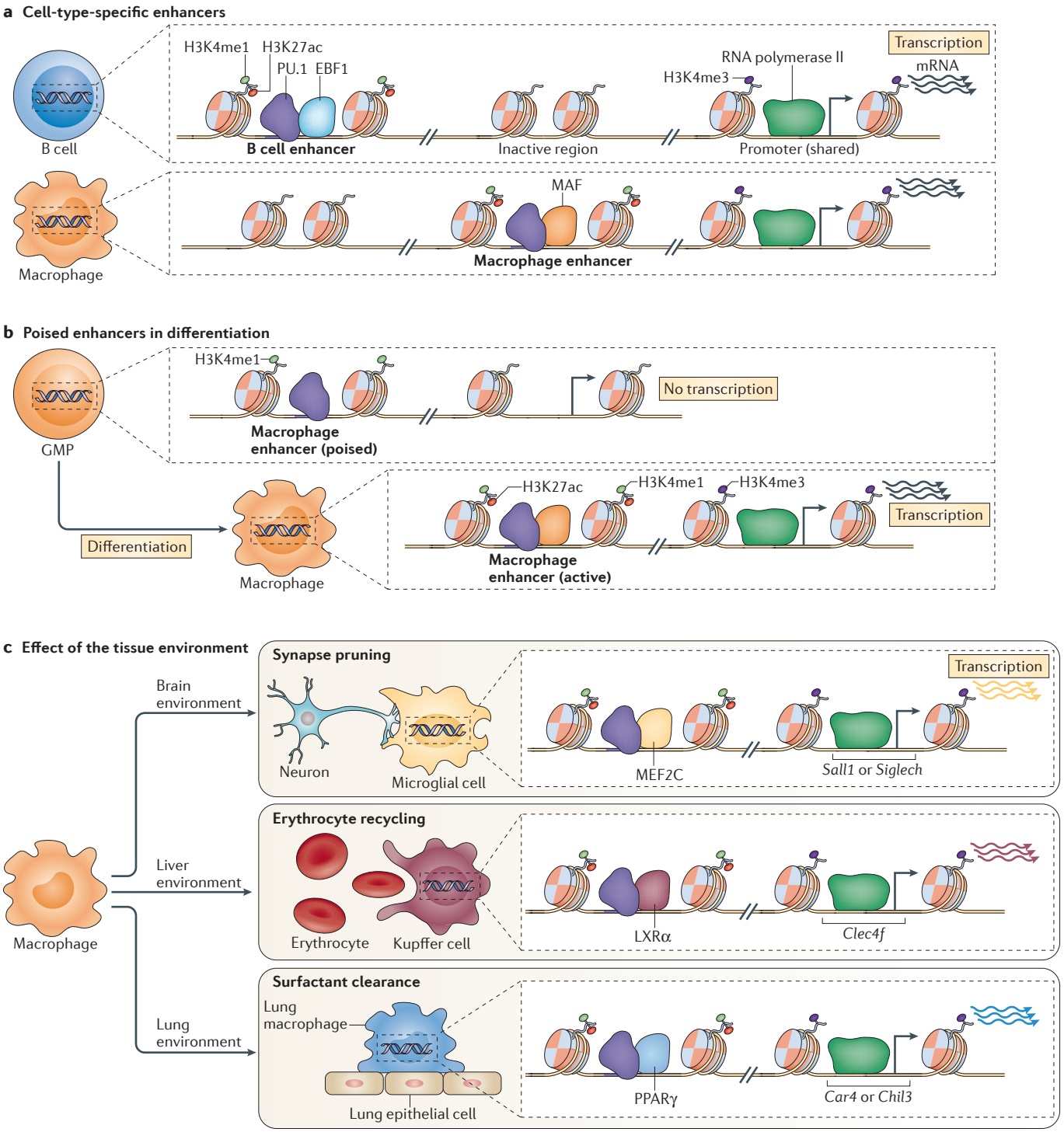


Figure 2 | **Multiple layers of chromatin regulation in immune cells.** **a** | Cell-type-specific enhancers. Alternative distal regulatory regions (enhancers) involved in lineage specification promote the transcription of the same gene in different cell types. The cell-type-specific transcription factors of B cells (blue) and macrophages (orange) bind in combination with PU.1 to active enhancers marked by histone 3 lysine 4 monomethylation (H3K4me1; green) and H3K27 acetylation (H3K27ac; red). Active promoters are indicated by H3K4me3 (purple) and RNA polymerase II binding. **b** | Poised enhancers in differentiation. Poised enhancers marked by H3K4me1 alone in the granulocyte–macrophage precursor (GMP) become active upon cell differentiation to induce transcription in macrophages through the co-binding of macrophage-specific transcription factors, such as MAF. **c** | Effect of the tissue environment. The tissue environment affects the regulatory landscape of a cell through the induction of specific transcription factors, leading to the expression of genes that are likely to be involved in the unique functional pathways of each tissue-specific cell type: *Sall1* and *Siglech* (sialic acid-binding immunoglobulin-like lectin H) for neuronal synapse pruning in microglia (brain-resident macrophages); *Clec4f* (C-type lectin domain family 4 member F) for erythrocyte recycling in Kupffer cells (liver-resident macrophages); and *Car4* (carbonic anhydrase 4) and *Chil3* for surfactant clearance in lung macrophages[14]. LXRα, liver X receptor-α; MEF2C, monocyte enhancer factor 2C; PPARγ, peroxisome proliferator-activated receptor-γ.

Fpr1 (formyl peptide receptor 1), *Cnlp* (also known as *Camp*) and *Saa3* (serum amyloid A3), to be prepared for future activation. By contrast, tolerizable genes, including *Il6* (interleukin-6), *Lipg* (endothelial lipase) and *Cspg4* (chondroitin sulphate proteoglycan 4), had a reduced response to restimulation with LPS[41]. Thus, the chromatin landscape offers clues about upcoming transitions in cell state and regulatory network.

Space: the tissue environment. Recent studies have shown that the chromatin landscape can also reflect another aspect of the cell: its local environment[13,14]. Most tissue-resident macrophages are established prenatally, develop in tandem with the organ in which they reside and are maintained in the adult[42]. It was recently shown that the tissue environment has a marked effect on the resulting function and identity of these macrophages (FIG. 2c). Specifically, analysis of tissue-specific enhancers in these cells revealed that signals from the local microenvironment shape the expression of unique tissue regulators — such as MEF2C in microglia, liver X receptor-α (LXRα) in Kupffer cells and peroxisome proliferator-activated receptor-γ (PPARγ) in peritoneal macrophages — and thereby globally programme the chromatin landscape[13,14]. For example, the presence of retinoic acid in the peritoneum triggers GATA-binding protein 6 (GATA6) binding to widespread enhancers that regulate the specific gene expression programme of peritoneal macrophages, including the transcription of *Tgfb2* (transforming growth factor-β2), *Serpinb2* (serine (or cysteine) peptidase inhibitor clade B member 2) and *Alox15* (arachidonate 15-lipoxygenase)[14,43]. The set of unique enhancers and the genes that they regulate in each tissue are likely to be responsible for emerging tissue-specific functions of each macrophage subpopulation; for example, synapse pruning by microglia, erythrocyte recycling by Kupffer cells and surfactant clearance by lung macrophages[44]. Indeed, many immune cell types, including regulatory T cells and natural killer cells, are known to infiltrate the diverse tissues of the body and take local residence[45,46]. Transcriptome studies suggest that these cells may also receive a local imprint, but a comparison of chromatin state is required for a better understanding of the environmental factors involved[45,46]. Notably, the observed plasticity of regulatory networks even within different populations of a single cell type demonstrates how chromatin profiling can determine the effect of the local environment on the gene regulatory network of a cell.

In this section on using chromatin profiling to infer a spatiotemporal cell view, we have described selected model systems in order to best illustrate the diversity of chromatin assays and how chromatin profiling data can be used to address various immunological questions — including how immune cells age, how haematopoiesis is regulated, how immune memory is encoded and how the environment affects immune function — in a manner that could not be accomplished by other approaches. In designing a specific research project, one must consider which assay or combination of assays is required to provide the temporal or spatial view of the regulatory network relevant to the research question.

Inferring transcription factor binding

Importantly, the chromatin landscape of a cell, which defines the functional *cis*-acting regulatory landscape, can also be used to determine the binding of *trans*-acting regulatory factors. ChIP–seq assays for specific transcription factors are used to identify their binding sites across the genome. However, each cell type expresses hundreds of transcription factors and it is not always clear which of these are most relevant. Transcription factors are often crucial in more than one cell type and are involved in distinct tasks depending on their binding partner: for example, interferon-regulatory factor 4 (IRF4) and IRF8 collaborate with the ETS transcription factors PU.1 and SPIB at ETS–IRF motifs (EICEs) in myeloid and B cells, but with BATF at AP-1–IRF motifs (AICEs) in T cells[47,48]. Without experimentally testing each transcription factor, the binding of specific transcription factors within *cis*-acting regulatory elements can be inferred from the presence of particular sequence motifs to which they bind and the 'footprint' that they leave in the pattern of chromatin accessibility[16,49–51]. When comparing B cells with T cells, OCT1-, MEF2- and GATA-binding motifs were found to be differentially enriched in the footprints of ATAC-seq peaks[16].

A special class of transcription factors function as pioneers that initiate chromatin accessibility to enable the subsequent binding of additional transcription factors[52,53]. These pioneer transcription factors, which in many cases are also lineage-determining transcription factors, have an important role in lineage specification by selecting the precise catalogue of genomic regions that form the cell's regulatory landscape[10,38,48,54,55]. Examples of pioneer factors that have been inferred from chromatin profiling in the haematopoietic system include GATA1 for the erythroid lineage, CCAAT/enhancer-binding protein-α (CEBPα) for the myeloid lineage and FOXO1 for the lymphoid lineage[15]. At the apex of the transcription factor hierarchy, pioneer factors can work in tandem with cell-type-specific transcription factors to programme the cell's chromatin landscape; in particular, PU.1 — the general haematopoietic cell pioneer factor — can bind with either B cell-specific transcription factors (such as EBF1 (also known as COE1)) or macrophage-specific transcription factors (such as MAF or IRF8) to designate alternative enhancers[10,14,56]. Likewise, cell-type-specific pioneer factors predetermine the enhancers that will recruit signal-dependent transcription factors to trigger a unique response to environmental and immune stimuli[9,55]. For example, a single enhancer regulating *Il1a* (interleukin-1α) expression in dendritic cells is bound by PU.1 and CEBPβ in unstimulated cells and joined by RELA and signal transducer and activator of transcription 1 (STAT1) following LPS stimulation[38]. Thus, by analysing the chromatin patterns and sequence of *cis*-acting regulatory elements, one can further deduce the temporal and spatial role of *trans*-acting factors.

Translating to human health

Arguably, the overarching goal of immunological studies is to learn more about the aetiology of human disease. Towards this aim, we have to translate what we know about methods and principles of chromatin dynamics in the mouse immune system into the study of human specimens. Much has already been learned from the preliminary studies of the Blueprint consortium, which was established to assay chromatin in human immune cells[57,58]. However, because of limitations in the type and amount of cells that can be obtained from human donors, it is often difficult to retrieve all of the cell types necessary for analysis and, with the exception of vaccinations[59,60], it is difficult to analyse samples following defined *in vivo* perturbations. Thus, many human studies are carried out in culture, but this inherently requires disturbing the natural environment of the cell. Accordingly, studies on primary cells from small animal models, commonly mice (such as those described above), are used to provide the basic principles for a working map of the mammalian immune system. Although most mouse immune cell types do have clear human counterparts, profiling the human epigenome is required for a more direct understanding of human disease, given

that the *cis*-acting regulatory landscape is a rapidly evolving feature[61]. In addition, human samples have the combined advantage and complication of being highly influenced by genetic variation. As exemplified in an elegant analysis of different mouse strains[62], the application of chromatin profiling across human immune cell types will enable genome-wide screening for the influence of causal variants in cell-type-specific enhancers. The high levels of genetic variation between individuals may create technical difficulties in terms of sequence alignment and may complicate characterization of the 'prototype' human epigenome. However, these differences can also be manipulated through comparative analysis and *in vitro* assays to elucidate the precise activity of regulatory elements and their role in disease.

With the aforementioned technological advances in chromatin profiling, we can now study large human cohorts to identify the regulatory regions that lead to disease (FIG. 3). Currently, an immense library of causal variants that affect countless diseases and phenotypes exists in the results of publicly available GWASs. However, the mechanisms through which these single-nucleotide polymorphisms (SNPs) influence gene expression are rarely known because the vast majority of causal variants implicated in disease and selection, such as those associated with animal domestication[63], are found outside of protein-coding sequences. In lymphoblastoid cell lines, studies found that sequence variation could lead to decreased chromatin accessibility and diminished RNA polymerase II-mediated transcription of nearby genes by disrupting the binding of transcription factors, such as CTCF and NF-κB family members, to *cis*-acting regulatory elements[64–66]. A high proportion of the SNPs affecting open chromatin — termed DNase hypersensitive quantitative trait loci (dsQTLs) — in lymphoblastoid cell lines were also classified as expression quantitative trait loci (eQTLs) and were associated with changes to the binding of transcription factors such as PU.1, basic leucine zipper transcription factor ATF-like (BATF), EBF1 and IRF4 (REF. 64). Connecting disease-causing sequence variants to their mechanism of action and the genes that they influence will help to decipher how they lead to disease and, thus, how to develop treatments. As in the previously described case of the *BCL11A* enhancer in erythroid cells, many of the regions in which disease-causing variants are found are specific to a certain cell type or condition, and thus their role in disease may be evident only when investigating the relevant immune cell type in the appropriate condition. A recent study compared GWAS-identified SNPs associated with autoimmune diseases with histone modification data in various immune cell types to link enhancer variants to disease and thereby discover their underlying mechanism[12]. The results show a significant overlap between disease-causing variants and *cis*-acting regulatory elements and can be used to provide clues as to the specific genes that are associated with disease in particular cell types. By integrating data on transcription factor binding sites, the precise disruption in the regulatory network can be inferred. In the example of *IKZF3*

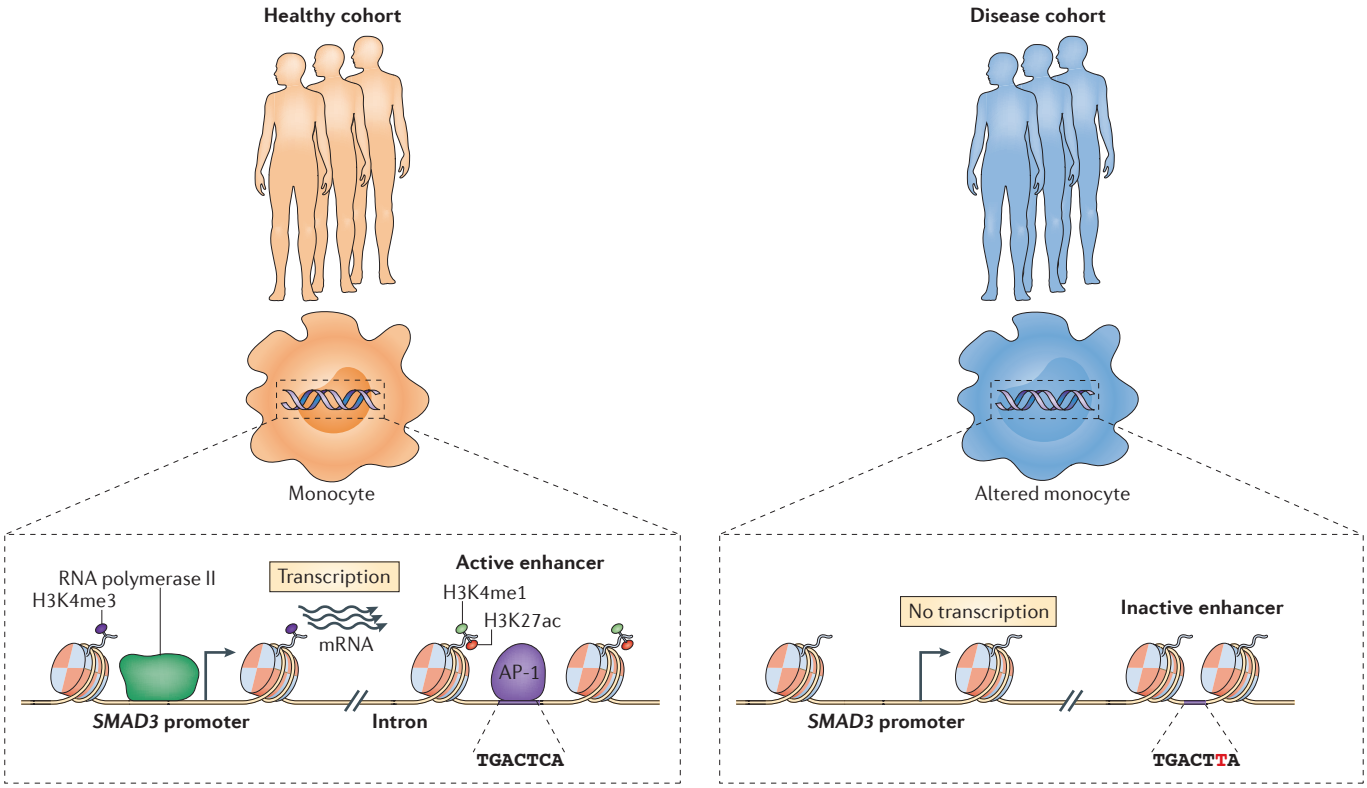


Figure 3 | **Association of human chromatin data and susceptibility to immune disease.** Cohort studies are designed to find sources of genetic variation between the control and disease groups, such as the single-nucleotide polymorphism (SNP) shown here located in an activator protein 1 (AP-1)-binding motif within the *SMAD3* intron (indicated by the red letter 'T'). This SNP, which is associated with Crohn disease, disrupts the binding of AP-1 to an enhancer that is active in healthy monocytes[12]. By comparing SNPs with chromatin profiles, we can determine whether they are located in regulatory elements on or near transcription factor-binding sites in the relevant cell type. The resulting disruption of the chromatin state leads to altered gene transcription and provides the mechanism of disease. H3K4me1, histone 3 lysine 4 monomethylation; H3K27ac, H3K27 acetylation; H3K4me3, H3K4 trimethylation.

mentioned above, the SNP associated with multiple sclerosis was located in a degenerate MEF2-binding motif within a B cell enhancer that is also bound by RELA (an NF-κB family member) and EBF1: in general, regions bound by MEF2, NF-κB and EBF1 coincided with genome-wide causal SNPs for multiple sclerosis[12]. Although the actual mutation fell outside the binding sites for RELA and EBF1, it is possible for mutations in the binding site of one transcription factor to indirectly affect the binding of nearby factors. Alternatively, the *IKZF3* SNP could function through as yet undefined regulatory mechanisms. With continued research along these lines, we will gain a better understanding of how human genetic variation leads to altered immune phenotypes and diseases. Applying chromatin analyses to clinical decisions will be complicated by the fact that each laboratory uses different protocols and analytical practices; we suggest that the results of different studies need to first be standardized to enable a meta-analysis of results that could be used for practical applications. We propose that any study of primary haematopoietic cells should include chromatin profiling with consideration of natural human variation.

Vision for the future

We believe that now is the time for chromatin profiling to be integrated as a key feature of immunological studies. Laboratories across the field will benefit from incorporating chromatin assays into their toolbox or collaborating with other researchers who already have. We have demonstrated the possible advantages of chromatin profiling to individual studies, but there is also a huge benefit to the community in general. The annotation of regulatory elements improves as chromatin profiles for each additional immune cell type are completed. From these combined data sets, we can produce a publicly available map of the regulatory landscape across the haematopoietic system in various species and tissues. Then, chromatin analysis will become as commonplace as expression analysis, with easily accessible databases used for reference and meta-analyses. In the future, researchers will be able to simply look up the enhancers regulating any given gene in any condition. Of course, these maps should be further substantiated and improved with functional assays, such as reporters for enhancer activity, knockout of specific chromatin remodellers (DNMTs, histone acetyltransferases and histone deacetylases) to determine their downstream effects, and clustered regularly interspaced short palindromic repeats (CRISPR)-mediated activation or repression of regulatory elements[38,67]. With these additional data, we will better understand the mechanism of gene regulation and how it can be influenced.

Progress in charting the gene regulatory networks of immune cells is also important for advancing other areas of science and medicine. The basic principles of gene regulation in development, differentiation, signalling and response that can be investigated in the relatively contained immune system will be widely applicable. As we identify the crucial elements involved in maintaining health, we can better understand the role of each cell population and how their dysregulation leads to disease. We can use these data to understand how one immune cell population might replace another malfunctioning one: for example, bone marrow cells may be transferred to the lungs to recapitulate the chromatin landscape and phenotype of lung macrophages[14,68]. Again, studying human cohorts is necessary to appreciate the level of inherent variation. The pharmaceutical industry will profit from the ability to link disease-causing SNPs with response to drug targets, leading to more effective and precise treatments. Autoimmune and inflammatory diseases, such as multiple sclerosis and rheumatoid arthritis, are highly heterogeneous. The clinical presentations of these diseases vary widely between patients, as does the response to current therapies. Drugs developed for rheumatoid arthritis and multiple sclerosis target different components of the immune system and include cytokine inhibitors and antibodies directed against various immune cells. Markers for disease classification, prognosis, therapeutic response and toxicity are lacking, as is a better understanding of the pathogenic mechanisms that initiate and perpetuate the disease. We predict that chromatin profiling techniques will eventually have a marked impact on how clinicians diagnose and target the appropriate causative cell type and pathway on a patient-by-patient basis. Instead of treating symptoms, or even changes in downstream gene expression, medication could be aimed at correcting the offending fault in the cell's regulatory network. Given the high value and information content of chromatin profiling and the recent development of cutting-edge technology, this technique is simply waiting to be exploited further.

Deborah R. Winter, Steffen Jung and Ido Amit are at the Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel. Correspondence to D.R.W. and I.A.

e-mails: deborah.winter@weizmann.ac.il; ido.amit@weizmann.ac.il

D.R.W. and I.A. contributed equally to this work.

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1. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 (2001).
2. Venter, J. C. *et al.* The sequence of the human genome. *Science* **291**, 1304–1351 (2001).
3. Heng, T. S., Painter, M. W. & Immunological Genome Project Consortium. The Immunological Genome Project: networks of gene expression in immune cells. *Nat. Immunol.* **9**, 1091–1094 (2008).
4. Kim, C. C. & Lanier, L. L. Beyond the transcriptome: completion of act one of the Immunological Genome Project. *Curr. Opin. Immunol.* **25**, 593–597 (2013).
5. Shay, T. & Kang, J. Immunological Genome Project and systems immunology. *Trends Immunol.* **34**, 602–609 (2013).
6. Barski, A. *et al.* High-resolution profiling of histone methylations in the human genome. *Cell* **129**, 823–837 (2007).
7. Schones, D. *et al.* Dynamic regulation of nucleosome positioning in the human genome. *Cell* **132**, 887–898 (2008).
8. Boyle, A. *et al.* High-resolution mapping and characterization of open chromatin across the genome. *Cell* **132**, 311–322 (2008).
9. Ostuni, R. *et al.* Latent enhancers activated by stimulation in differentiated cells. *Cell* **152**, 157–171 (2013).
10. Heinz, S. *et al.* Simple combinations of lineage-determining transcription factors prime *cis*-regulatory elements required for macrophage and B cell identities. *Mol. Cell* **38**, 576–589 (2010).
11. Bauer, D. E. *et al.* An erythroid enhancer of *BCL11A* subject to genetic variation determines fetal hemoglobin level. *Science* **342**, 253–257 (2013).
12. Farh, K. K.-H. *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* **518**, 337–343 (2015).
13. Gosselin, D. *et al.* Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* **160**, 351–352 (2014).
14. Lavin, Y. *et al.* Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* **159**, 1312–1326 (2014).
15. Lara-Astiaso, D. *et al.* Chromatin state dynamics during blood formation. *Science* **345**, 943–949 (2014).
16. Buenrostro, J., Giresi, P., Zaba, L., Chang, H. & Greenleaf, W. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat. Methods* **10**, 1213–1218 (2013).
17. Thurman, R. *et al.* The accessible chromatin landscape of the human genome. *Nature* **489**, 75–82 (2012).
18. Cusanovich, D. A. *et al.* Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing. *Science* **348**, 910–914 (2015).
19. Greenleaf, W. J. Assaying the epigenome in limited numbers of cells. *Methods* **72**, 51–56 (2015).
20. Cedar, H. & Bergman, Y. Epigenetics of haematopoietic cell development. *Nat. Rev. Immunol.* **11**, 478–488 (2011).
21. Broske, A.-M. *et al.* DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat. Genet.* **41**, 1207–1215 (2009).
22. Ji, H. *et al.* Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature* **467**, 338–342 (2010).
23. Shlush, L. I. *et al.* Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* **506**, 328–333 (2014).
24. Xie, W. *et al.* Epigenomic analysis of multilineage differentiation of human embryonic stem cells. *Cell* **153**, 1134–1148 (2013).
25. Sun, D. *et al.* Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. *Cell Stem Cell* **14**, 673–688 (2014).

26. Giresi, P., Kim, J., McDaniell, R., Iyer, V. & Lieb, J. FAIRE (formaldehyde-assisted isolation of regulatory elements) isolates active regulatory elements from human chromatin. *Genome Res.* **17**, 877–885 (2007).
27. Heintzman, N. *et al.* Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* **39**, 311–318 (2007).
28. Creyghton, M. *et al.* Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl Acad. Sci. USA* **107**, 21931–21936 (2010).
29. Lieberman-Aiden, E. *et al.* Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289–293 (2009).
30. Sanyal, A., Lajoie, B., Jain, G. & Dekker, J. The long-range interaction landscape of gene promoters. *Nature* **489**, 109–113 (2012).
31. Kieffer-Kwon, K.-R. *et al.* Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. *Cell* **155**, 1507–1520 (2013).
32. Cui, K. *et al.* Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. *Cell Stem Cell* **4**, 80–93 (2009).
33. Bernstein, B. E., Mikkelsen, T. S., Xie, X. & Kamal, M. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326 (2006).
34. Rada-Iglesias, A. *et al.* A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* **470**, 279–283 (2010).
35. Odink, K. *et al.* Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature* **330**, 80–82 (1987).
36. Lam, M. T. Y. *et al.* Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. *Nature* **498**, 511–515 (2013).
37. Treiber, T. *et al.* Early B cell factor 1 regulates B cell gene networks by activation, repression, and transcription- independent poising of chromatin. *Immunity* **32**, 714–725 (2010).
38. Garber, M. *et al.* A high-throughput chromatin immunoprecipitation approach reveals principles of dynamic gene regulation in mammals. *Mol. Cell* **47**, 810–822 (2012).
39. Monticelli, S. & Natoli, G. Short-term memory of danger signals and environmental stimuli in immune cells. *Nat. Immunol.* **14**, 777–784 (2013).
40. Netea, M. G., Quintin, J. & van der Meer, J. W. M. Trained immunity: a memory for innate host defense. *Cell Host Microbe* **9**, 355–361 (2011).
41. Foster, S., Hargreaves, D. & Medzhitov, R. Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* **447**, 972–978 (2007).
42. Ginhoux, F. & Jung, S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* **14**, 392–404 (2014).
43. Okabe, Y. & Medzhitov, R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell* **157**, 1–13 (2014).
44. Varol, C., Mildner, A. & Jung, S. Macrophages: development and tissue specialization. *Annu. Rev. Immunol.* **33**, 643–675 (2015).
45. Burzyn, D., Benoist, C. & Mathis, D. Regulatory T cells in nonlymphoid tissues. *Nat. Immunol.* **14**, 1007–1013 (2013).
46. Yokoyama, W. M., Sojka, D. K., Peng, H. & Tian, Z. Tissue-resident natural killer cells. *Cold Spring Harb. Symp. Quant. Biol.* **78**, 149–156 (2013).
47. Glasmacher, E. *et al.* A genomic regulatory element that directs assembly and function of immune-specific AP-1–IRF complexes. *Science* **338**, 975–980 (2012).
48. Ciofani, M. *et al.* A validated regulatory network for Th17 cell specification. *Cell* **151**, 289–303 (2012).
49. Boyle, A. *et al.* High-resolution genome-wide *in vivo* footprinting of diverse transcription factors in human cells. *Genome Res.* **21**, 456–464 (2011).
50. Pique-Regi, R. *et al.* Accurate inference of transcription factor binding from DNA sequence and chromatin accessibility data. *Genome Res.* **21**, 447–455 (2011).
51. Neph, S. *et al.* An expansive human regulatory lexicon encoded in transcription factor footprints. *Nature* **489**, 83–90 (2012).
52. Zaret, K. S. & Carroll, J. S. Pioneer transcription factors: establishing competence for gene expression. *Genes Dev.* **25**, 2227–2241 (2011).
53. Cirillo, L. & Zaret, K. An early developmental transcription factor complex that is more stable on nucleosome core particles than on free DNA. *Mol. Cell* **4**, 961–969 (1999).
54. Ostuni, R. & Natoli, G. Lineages, cell types and functional states: a genomic view. *Curr. Opin. Cell Biol.* **25**, 759–764 (2013).
55. Winter, D. R. & Amit, I. The role of chromatin dynamics in immune cell development. *Immunol. Rev.* **261**, 9–22 (2014).
56. Vilagos, B. *et al.* Essential role of EBF1 in the generation and function of distinct mature B cell types. *J. Exp. Med.* **209**, 775–792 (2012).
57. Adams, D. *et al.* BLUEPRINT to decode the epigenetic signature written in blood. *Nat. Biotechnol.* **30**, 224–226 (2012).
58. Martens, J. H. A. & Stunnenberg, H. G. BLUEPRINT: mapping human blood cell epigenomes. *Haematologica* **98**, 1487–1489 (2013).
59. Pulendran, B. Systems vaccinology: Probing humanity's diverse immune systems with vaccines. *Proc. Natl Acad. Sci. USA* **111**, 12300–12306 (2014).
60. Brodin, P. *et al.* Variation in the human immune system is largely driven by non-heritable influences. *Cell* **160**, 37–47 (2015).
61. Ballester, B. *et al.* Multi-species, multi-transcription factor binding highlights conserved control of tissue-specific biological pathways. *eLife* **3**, e02626 (2014).
62. Heinz, S. *et al.* Effect of natural genetic variation on enhancer selection and function. *Nature* **503**, 487–492 (2013).
63. Rosengren Pielberg, G. *et al.* A *cis*-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nat. Genet.* **40**, 1004–1009 (2008).
64. Degner, J. *et al.* DNase I sensitivity QTLs are a major determinant of human expression variation. *Nature* **482**, 390–394 (2012).
65. Kasowski, M. *et al.* Variation in transcription factor binding among humans. *Science* **328**, 232–235 (2010).
66. McDaniell, R. *et al.* Heritable individual-specific and allele-specific chromatin signatures in humans. *Science* **328**, 235–239 (2010).
67. Mikkelsen, T. *et al.* Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553–560 (2007).
68. Suzuki, T. *et al.* Pulmonary macrophage transplantation therapy. *Nature* **514**, 450–454 (2014).
69. Richmond, T. & Davey, C. The structure of DNA in the nucleosome core. *Nature* **423**, 145–150 (2003).
70. Felsenfeld, G. & Groudine, M. Controlling the double helix. *Nature* **421**, 448–453 (2003).
71. Kouzarides, T. Chromatin modifications and their function. *Cell* **128**, 693–705 (2007).
72. Gross, D. S. & Garrard, W. T. Nuclease hypersensitive sites in chromatin. *Annu. Rev. Biochem.* **57**, 159–197 (1988).
73. Amendola, M. & Van Steensel, B. Mechanisms and dynamics of nuclear lamina–genome interactions. *Curr. Opin. Cell Biol.* **28**, 61–68 (2014).
74. Grewal, S. & Moazed, D. Heterochromatin and epigenetic control of gene expression. *Science* **301**, 798–802 (2003).
75. Dixon, J. *et al.* Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376–380 (2012).
76. Rao, Suhas, S. P. *et al.* A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665–1680 (2014).
77. Johnson, D. S., Mortazavi, A., Myers, R. M. & Wold, B. Genome-wide mapping of *in vivo* protein-DNA interactions. *Science* **316**, 1497–1502 (2007).

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Competing interests statement

The authors declare no competing financial interests.

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