Development of multicellular organisms requires the differential usage of our genetic information to change one cell fate into another. This process drives the appearance of different cell types that come together to form specialized tissues sustaining a healthy organism. In the last decade, by moving away from studying single genes toward a global view of gene expression control, a revolution has taken place in our understanding of how genes work together and how cells communicate to translate the information encoded in the genome into a body plan. The development of hematopoietic cells has long served as a paradigm of development in general. In this review, we highlight how transcription factors and chromatin components work together to shape the gene regulatory networks controlling gene expression in the hematopoietic system and to drive blood cell differentiation. In addition, we outline how this process goes astray in blood cancers. We also touch upon emerging concepts that place these processes firmly into their associated subnuclear structures adding another layer of the control of differential gene expression.

Introduction

Hematopoiesis is one of the best understood developmental pathways [1,2] and has extensively been studied in mice. The origin of blood cell development in the embryo is the mesodermal germ layer in the mammalian embryo, and hematopoietic specification occurs in two waves: The first wave takes place in the extraembryonic blood islands of the yolk sac and gives rise to primitive progenitor cells with mostly erythroid and myeloid potential [3,4]; the second wave gives rise to definitive hematopoietic stem cells (HSCs) and takes place at the ventral part of the dorsal aorta in the aorta–gonad–mesonephros (AGM) region of the embryo [5]. Cells emerging during the second wave migrate first to the fetal liver and later to the bone marrow. Here, they are maintained in a specialized niche and are largely quiescent, and if growing, either self-renew or enter differentiation to sustain mature blood cell production throughout lifetime. All HSCs are born from a specialist endothelial cell layer, the hemogenic endothelium (HE), which communicates with the dorsal mesenchyme. In response to signals, HE cells undergo a cellular shape transition, the endothelial–hematopoietic transition (EHT), forming intra-aortic clusters, which undergo several maturation steps before floating off into the bloodstream. Blood cell development therefore involves a carefully regulated cascade of gene expression changes that are regulated by molecular mechanisms linking genomic responses to a multitude of signals coming from the outside. It matters, where a cell has been and who it has talked to. In turn, it matters whether a cell is responsive to an outside signal, making development
and differentiation an intricate, but highly robust balancing act that occurs in multiple cells at the same time. This review will summarize seminal studies, which uncovered the players involved in this balancing act and highlight the notion that signaling-responsive transcription regulation and chromatin dynamics are at the heart of the mechanisms maintaining and changing cellular identity. We will also highlight that perturbing any of these mechanisms leads to a disturbance of differentiation and, in some cases, to the development of malignant cells that have opted out of normal growth and differentiation control.

**Transcription factors control blood cell development and differentiation**

Blood cell lineage-specific gene expression is under the control of specific transcription factors. A large number of studies employing genetically modified mice showed that the absence of lineage specifically expressed factors leads to a perturbation of differentiation or a complete absence of the respective lineage. One of the first examples of a knockout removing an important TF was that of the erythroid-specifically expressed TF GATA1, which led to a complete absence of erythroid (and megakaryocytic) differentiation, while other lineages appeared to be unperturbed [6]. The underlying molecular mechanism of differentiation defects in the absence of a lineage-determining TF is the deregulation of genes carrying binding sites for this TF. Knockout experiments also highlighted the fact that TFs act in a hierarchical fashion. The elimination of earlier acting TFs such as TAL1 or RUNX1 affects HSC formation and thus the development of the entire hematopoietic system, whereas elimination of others such as PU.1 largely affects the myeloid and B-cell lineages. Recent studies using single-cell RNA-seq approaches have visualized the successive activation of specific developmental trajectories by performing ‘nearest neighbor’ analyses, which determine changes in the gene expression patterns of single cells and order them according to the direction of increased maturation. Such analyses highlight the different branches of the hematopoietic system and show that they deviate from each other earlier than previously thought [7,8]. Performing such studies with hematopoietic cells lacking a lineage-determining factor clearly showed the absence of specific branches in the trajectory [9]. Another hallmark of the hierarchical action of TFs is the finding that they are often only critically required at specific stages of development even if still expressed at other stages. Examples for this notion are again RUNX1 and TAL1, and the removal of their genes from the germ line strongly blocks HSC development, but when removing it conditionally after HSCs have formed, their maintenance is not affected [10–12].

However, such a clear-cut result is not seen with all TF knockouts and the reason for this behavior is the fact that TFs operate within large interacting protein assemblies as explained in further detail below. TFs have a modular structure with different domains that fulfill different functions and interact with different proteins. Recent studies showed that crippling TFs by removing individual domains can have unexpected effects that shed light on their actual function in gene regulation and point to an amazing robustness of protein complex formation driving gene expression. An example is again the transcription factor TAL1. Deletion of the whole factor abolishes HSC emergence completely, but deletion of the DNA-binding domain alone has a much milder phenotype and factor binding can be detected at a subset of genomic targets [13]. A different result was observed after removing the DNA-binding domain from the ubiquitously expressed TF SP1. This mutation affects all developmental pathways, and a germ-line mutation is an embryonic lethal [14]. However, in contrast to lineage-determining factors, removing it conditionally later in development had very little effect [15]. The explanation for this finding came with the analysis of the differentiation of mouse embryonic stem cells into blood precursors *in vitro*, which showed that the knockout still expressed a truncated protein and that the effect of the mutation on gene expression was cumulative. The full knockout of the *Sp1* gene was incompatible with differentiation and so was the full deletion of the *Sp1* orthologue *Sp3* in a *Sp1* hypomorphic genetic background, indicating that the truncated version of *Sp1* needed *Sp3* to function. During the differentiation of cells expressing a truncated Sp1, bulk gene expression patterns of purified cells became more and more diverse. Single-cell RNA-seq experiments and the analysis of differentiation trajectories of such cells showed why this was the case: Cells entered the correct gene expression trajectory, but seemed to do this at different time points, forming transcriptionally diverse cell populations. In essence, cells do not execute cell fate decisions as a cohort, meaning that robustness of differentiation was lost [15]. However, the system could only tolerate a certain level of deregulation: Once past the progenitor stage, differentiation crashed, and mutant cells were unable to form terminally differentiated blood cells [16]. A break-down of robustness of differentiation can also be seen when another crucial level of control of differentiation is disturbed: the expression of correct TF levels. Many crucial TFs show haploinsufficiency
phenotypes when one genetic copy is deleted or expression levels are reduced by the mutation of important cis-regulatory elements, with the kinetics of development being perturbed. This notion is true for GATA2 whose downregulation by the mutation of an essential enhancer [17] or by haploinsufficiency [18] causes various hematopoietic defects and predisposes to leukemia. The latter is also true for a cis-regulatory mutation of the gene encoding PU.1, SPI1 [19]. Last, but not least, RUNX1 needs to be expressed at carefully controlled levels to drive hematopoietic differentiation and specification [20,21].

Taken together, these studies show that the effects of the crippling of an essential TF or its gene on gene expression control have to be seen within the context of development being a dynamic and highly robust process. The system is composed of large interacting protein assemblies and partly redundant components, which compensate until they fall apart or are malfunctioning, meaning that the defect occurs way before phenotypic alterations can be seen. The current challenge is to identify the point when this occurs. This notion will become important when trying to interpret how mutant transcription factors set differentiating cells on the path to cancer.

Transcription factors collaborate and respond to signals

TFs come in families that bind to specific DNA-binding motifs within cis-regulatory elements such as enhancers and promoters, which are responsible for determining how a gene is regulated and when and in which cell type it is expressed. Each regulatory region contains multiple TF-binding motifs, which often are highly conserved depending on the nature of a gene and whether its function is conserved in evolution. A good example is the ‘Heptad’, a consortium of co-localizing transcription factors such as GATA2, TAL1, RUNX1, and FLI1 and the bridging factors LDB1/LMO2 that specify the cis-regulatory elements of genes expressed in hematopoietic stem and progenitor cells [22,23]. The spatial arrangement of TF-binding sites is often not conserved [24], but there are exceptions with TFs that directly interact on DNA and whose binding is interdependent. Here, the spacing of binding motifs can be very precise, as, for example, seen with the pair AP-1/TEAD in the hemogenic endothelium [25], the pair RUNX1/ETS1 [26], or the GATA/E-Box motifs within the heptad [27].

As exemplified by SP1, ubiquitously expressed TFs cooperate with tissue-specific factors to set up differential gene expression patterns, and thus, the binding patterns of TFs are highly specific for each cell type [28]. Importantly, binding patterns are highly dynamic and can be maintained in self-renewing cells [29] or change during development [30,31]. In this context, it is noteworthy that transcription is not a uniform process but occurs in bursts that are regulated by the burst frequency, indicating that genes are in intricate contact with their environment [32,33]. The cell receives signals from various sources, which trigger developmental changes and are integrated within the genome by the action of inducible and signaling-responsive TFs. Many of these factors can be activated in all cells and include the AP-1 (JUN/FOS) factor families, which respond to MAP kinase signaling [34], STATs responding to cytokine receptor signaling [35], SMADs mediating TGF-β signaling [36,37], TEAD/YAP mediating Hippo signaling [38], or NFAT family members (linked to Ca++-signaling) [39]. As a result of the activation of such factors, enhancer elements can be activated de novo, or become more active, driving increased levels of gene expression. However, note that also noninducible TFs present can be regulated in their activity by signaling-dependent post-translational modifications such as phosphorylation with RUNX1 being a prominent example [40].

While we have a fairly good idea about what regulates the activity of inducible transcription factors, we know very little about how the different modes of signal transmission interplay with each other across the genome. This notion becomes important when different signals are being integrated at the genome level by regulating TF binding. For example, the abolition of AP-1 binding during hematopoietic specification by using a dominant-negative version of FOS led to a loss of binding of the Hippo signaling-responsive factor TEAD at the composite genomic sites described above [25]. Hippo signaling is activated by the onset of blood flow, which creates biomechanical forces stimulating Rho-GTPase signaling [41]. During T-cell activation, MAP kinase and Ca++ signaling are integrated by a cooperation of AP-1 and NFAT and at a specific subset of sites with composite-binding motifs one factor cannot bind without the other [42,43], thus ensuring that genes respond only when both signals are present. These few examples show that we are only now starting to obtain a glimpse of the principles and staggering complexity of how the multitude of signaling inputs that a cell encounters are integrated within the genome and shape a genomic response.

TFs can both activate and repress gene expression and form dynamic gene regulatory networks

A large number of Zn²⁺ finger TFs are bona fide repressors with REST, a factor that is required to
Transcriptional control of hematopoiesis

B. Edginton-White and C. Bonifer

Transcriptional control of hematopoiesis

Transcription factors interact with a specific chromatin landscape

The most important feature of TFs is that they recognize specific DNA sequences and therefore are able to read the genetic code. However, within the eukaryotic
nucleus they encounter a formidable obstacle to this process in the form of chromatin. Here, DNA is wrapped around nucleosomes, which are then packed into higher-order structures of differential compaction, depending on whether the genes within these structures are active, potentially active, or stashed away in heterochromatin. In order for the genetic code to be accessed by TFs, chromatin needs to be remodeled and modified, which is achieved by different mechanisms. One mechanism is the opening of chromatin by pioneer factors, which are capable of binding to nucleosomal DNA and then cooperate with other factors to nucleate a transcription factor complex [58]. Other TFs interact with nucleosomes in different ways with most factors binding the nucleosomal linker regions [59]. All binding modes have in common that after a stable TF assembly is established, TFs recruit chromatin remodelers such as SWI/SNF complexes that use ATP to ‘peel’ DNA off the nucleosome and free up sequences for further binding [60]. A variant of the second mechanism is ‘assisted loading’ whereby an inducible factor binds, recruits chromatin remodelers that enable the binding of a second factor which cannot normally bind, and then leaves again, leaving stably remodeled and TF-bound chromatin behind [61,62]. During the assembly process, TF complexes recruit further cofactors such as histone acetyltransferases (HATs) that facilitate transcription by modifying the N-terminal tails of the surrounding nucleosomes and stabilize an open chromatin structure that is devoid of nucleosomes and exists as a nuclease hypersensitive site [63]. Given the importance of chromatin remodelers and modifiers in gene activation, it does not come as a surprise that these proteins are essential components of the regulatory machinery driving hematopoiesis [64–66].

The establishment of stable TF complexes and modified chromatin is not the only mechanism that is required to activate transcription. TF and cofactor complexes at the different enhancers and the promoter of a gene contact each other within nuclear space [67] and form large protein–DNA complexes on cis-regulatory elements that contain all the factors necessary to activate mRNA synthesis by RNA polymerase, and form a regulatory unit or chromatin hub [68]. The architecture of such units can be simple or complex—depending on the complexity of gene regulation during development and in different tissues [69,70]. The reason for such complexity is that during development, genes can be regulated by a relay of differentially/tissue-specifically active cis elements. A good example for this notion is the chicken lysozyme locus, which is expressed in the oviduct or in macrophages and uses different and shared tissue-specific elements and factors to drive different regulatory modes of gene expression [71]. Moreover, even genes that are expressed in every cell, that is, ‘housekeeping genes’, are regulated by a relay of different factors thus keeping chromatin

---

**Fig. 1.** Development involves the alterations of gene regulatory networks. (A). Inferring regulatory relationships between transcription factor genes by perturbation of the expression of one factor and measuring the gene expression response of all genes. Note that such experiments require multiple measurements to identify statistically significant correlations. (B) Identifying regulatory relationships by direct binding experiments as described in Ref. [119]. This includes digital footprinting, which identifies factor families binding to the same motif or identifying the precise factor by using chromatin immunoprecipitation assays. Identified binding sites are then annotated to their rightful promoter using promoter capture HiC (chromosome conformation capture). Compared with gene expression analysis, this strategy allows to identify the differential wiring of GRNs in different cell types as depicted in C and D. Genes bound by the different TFs or TF families are depicted as rectangles with the color highlighting their expression level as indicated in the figure.
open and ensuring their sustained activity [31]. The latter mechanism highlights several important concepts in gene regulation: An active, transcriptionally permissive chromatin structure has to be actively maintained. In the absence of activators, an inactive chromatin structure is established by repressing factors such as DNA methyltransferase and histone deacetylases, which methylate DNA and remove the acetylation mark from histones. Secondly, an active chromatin pattern that is nuclease accessible and carries active histone marks is cell-type specific. Finally, it is not the promoters, but the nonpromoter elements that contain the information of tissue-specific gene expression and mirrors tissue-specific gene expression patterns [72,73].

Each transcription cycle is regulated by the balance of activating and repressing factors responding to outside signals [74]. A large number of genes maintain their transcriptionally active structure throughout cell division. However, during mitosis, TF complexes are largely stripped off chromatin and the question arises how they reform. It is now clear that the parent set of modified histones are distributed to the two daughter strands. Modification patterns are therefore retained during mitosis and mark genes that are activated after mitosis [75]. It is also clear that certain TFs, such as FOXA1, are capable of binding to mitotic chromatin and form the basis of re-assembled TF complexes creating an active chromatin structure once the nuclear environment has been reformed [74,76]. This transcriptional memory is often dependent on signaling processes, as shown during the formation of T-cell memory: Stable TF binding allowing rapid reactivation of genes by a second stimulus is dependent on the constant reinforcement of factor binding by cytokine signaling, employing inducible TFs. The absence of cytokine signaling leads to a loss of an active and transcriptionally permissive chromatin structure [77]. A similar transcriptional memory is also established in macrophages after a first inflammatory stimulus [78]. In a developmental context, this interplay of inducible and constitutive factors establishing an early memory of a previously received signal, also referred to as priming [79], plays a decisive role in changing or maintaining cell identities, as exemplified by neuronal development of C. elegans. The developmental timing of regulation of the Lsy-6 miRNA locus is dictated by a NOTCH responsive an early enhancer. Those neuronal precursor cells receiving the signal upregulate the gene earlier as compared to those who did not with a strong impact on gene expression patterns. The result is a functional left–right asymmetry in otherwise morphologically symmetric neurons [80]. Developing blood cells are embedded in a sea of signals that have a profound impact on gene expression. One of the challenges in the next years will be to unravel the order of events of how genes are activated in hematopoietic development and how external signals such as soluble factors, mechanical forces, and spatial context regulate the ordered formation of HSCs, cells of the different hematopoietic lineages, and hematopoietic tissues such as the thymus and lymph nodes. Single-cell analyses of chromatin changes and expression patterns in developing cells together with spatial information will be crucial to answer these questions [9,81]. Such studies need to be combined with studies of surface molecule mapping [82] and the analysis of intracellular signaling processes using advanced imaging—a formidable task.

Gene regulatory processes take place in different parts of the nucleus

Gene regulation cannot be viewed without taking into account where it takes place—in the nucleus (Fig. 2). In recent years, it has become clear that this organelle displays a highly organized structure, with genes occupying different compartments depending on their activity state, the nature of their neighbors, and whether they are transiently or permanently silenced [83–85]. The latter distinction is important, because transcription can be rapidly switched off with genes remaining in a poised state ready for further activation of repression, which is mediated by polycomb-repressive complexes (PRCs). PRC complexes come as two general types, PRC1 and PRC2. PRC2 contains the EZH1/2 methyltransferase, which deposits methyl groups on histone H3K27. H3K27me3 binds the PRC1 complex, which then ubiquitinates histone H2A at target promoters resulting in a block of transcriptional elongation by RNA polymerase II, with the nonelongating form of RNA polymerase still being associated at these sites [86,87]. PRC complexes at promoters interact with each other in nuclear space and form a long-range network of transcriptionally silent genes [88,89] that can intermingle with active genes [90] to rapidly switch from one state to another. In contrast, true heterochromatic regions such as centromeres, telomeres, repeat elements, and genes that are stably silenced display a highly compact chromatin structure, are not bound by RNA PolIII, and are associated with the nuclear periphery and the nuclear lamina [91].

Another level of chromosomal organization of higher eukaryotes, which is associated with differential gene expression, are topologically associated domains (TADs) [92,93]. TADs partition chromosomes into regulatory domains inhibiting interactions between neighboring chromosomal regions. TADs are in average
In the last few years, another feature within the nucleus has caught attention—that of nuclear speckles or membrane-less organelles (MLOs). Such structures can be formed by liquid–liquid phase separation (LLPS), which in biological systems is essentially a process that is based on an interaction of molecules that excludes water [99]. The nucleus contains a multitude of such structures [100]. The best known are nuclear speckles, which are the sites of splicing, and the nucleolus, which is the site of rRNA synthesis that originates from multiple repeats of rDNA genes. RNA itself is sufficient to nucleate the formation of a nucleolus, which is faithfully reformed after cell division [101]. Transcriptionally inactive heterochromatin containing the heterochromatin protein HP1 consists of another nuclear compartment at the nuclear periphery, which protects the genome from mechanical stress [102]. Proteins, such as HP1, are capable to form condensates by themselves [103] and a tell-tale sign of their ability to do so are domains of intrinsically disordered regions that appear to be devoid of structure but are essential for phase separation [104]. A large number of TFs, including those important for hematopoietic differentiation processes [105,106], contain such regions and are able to form large assemblies without having to be too selective and sprout-specific domains for every possible interaction [107]. Under physiological salt condition, unmodified chromatin undergoes phase separation in vitro or when injected.
into the nucleus, and this feature is modified by histone acetylation and protein binding [108]. A large number of factors contribute to a specific speckle type indicating that such structures play a global role in organizing nuclear processes [109]. Transcription is no exception. Microscopic analysis had shown many years ago that RNA polymerase II is organized in foci within the nucleus and appears to occur at fixed sites called ‘transcription factories’ [110]. More recently, the partition of transcriptional processes into separate assemblies was revived with the advent of global chromatin immunoprecipitation assays that uncovered that genes with complex regulatory regions (also termed ‘superenhancers’) form large, DNA-dependent molecular assemblies. It was suggested that these assemblies are able to undergo phase transition, thus forming regulatory entities with their own rules [111,112]. Moreover, it was also suggested that RNA polymerase II can shuttle between a transcription and a splicing compartment depending on its phosphorylation status [113]. However, while it is clear that such protein–DNA assemblies containing TFs and their cofactors form condensates in vitro and speckles in vivo, there is still some controversy whether DNA-dependent factor assembly represents true LLPS in living cells [114,115]. Nevertheless, it is now clear that compartmentalization is an essential part of regulatory processes within the nucleus, which drives the behavior of proteins in terms of their assembly kinetics and activity of enzymes. The challenge in the next years will be to precisely define the role of each compartment and which factors are involved in deciding how genes choose where to go to and are involved in driving compartmentalization.

The malignant state—differentiation going sideways

It is now clear that all of the mechanisms described in this review so far are important for normal development. Decades of research using knockout mice have shown that the machinery regulating differential gene expression is highly robust with a high inbuilt level of redundancy. However, they also showed that defects do not always manifest themselves immediately but can appear later in the life of an organism in the form of cancer, which is exemplified by certain types of blood cancers, occurring in families with inherited mutations in TF genes [116] that predispose patients to acute myeloid leukemia (AML). However, most cancer-causing mutations occur as somatic mutations in early hematopoietic precursor and stem cells. Recurrent mutations are seen in genes controlling gene regulation and epigenetic processes impacting cell fate decisions [117]. This involves genes encoding TFs (i.e., RUNX1 or C/EBPα), chromatin remodelers and modifiers (i.e., CHD4, CBP), polycistron family members (i.e., EZH2), DNA methyltransferases (i.e., DNMT3A) but also demethylases such as TET1/2. Moreover, we also find mutations in genes encoding signaling molecules controlling gene expression driving growth such as RAS, genes encoding architectural proteins such as CTCF, and genes encoding splicing factors. AML is mostly a disease of the elderly, with mutations in genes encoding transcriptional and epigenetic regulators occurring first, which are then followed by additional mutations in growth-promoting genes [118,119]. Such successive acquisition of mutations first generates progenitor cells with slightly impaired differentiation capacity, which manifests itself as clonal hematopoiesis where the normally tightly regulated balance of differentiation is disturbed. One particular progenitor clone expands and contributes excessively to blood cell development without causing any overt disease phenotype. However, the seed is then laid for secondary mutations, which then lead to a complete impediment of differentiation and excessive malignant growth. It is now clear that different driver mutations have a different impact on the differentiation trajectory and the epigenetic landscape. As a result of a defect in an important regulator of cell fate driving a normal developmental trajectory, malignant cells adopt new identities distinct from normal cells and differentiation goes ‘sideways’ [120,121] (Fig. 3). The question now arises, what is the nature of these new cellular identities and how are they maintained as compared to normal cells. Normal cellular differentiation processes have been shaped and perfected by evolution over millions of years, whereas malignant cell differentiation is a product of patient-specific clonal selection that occurs in a much smaller time frame: Being ‘imperfect’, the question arises of why are malignant cells so difficult to eradicate?

The answer to this question lays in the robustness and plasticity of the differentiation process, that is, life itself. Similar to normal cells, malignant cells are maintained by distinct GRNs that drive common and AML subtype-specific signaling and metabolic pathways. It should be noted that while cancers come in different forms and can arise from many tissues, the rewiring of normal GRNs into one that sustains a malignant phenotype is a hallmark of all of them. In AML, each mutation shapes the aberrant differentiation process in a different way, and even different mutations in a single TF-encoding gene such as RUNX1, which give rise to different aberrant version of RUNX1, can lead to completely different disease outcomes and cellular...
identities with distinct chromatin landscapes [122,123]. Moreover, the inducible expression of different RUNX1 oncoproteins causes an immediate reprogramming of their chromatin and TF-binding landscape, which is specific for each aberrant protein [123,124]. These data suggest that once different epigenetic landscapes have been set up after the first oncogenic hit, cells on their way to malignancy tweak their GRNs to compensate for the weakness of one differentiation process to activate another to maintain a stable state that is compatible with growth. We find the aberrant activation of genes encoding lineage-inappropriate TFs, which then become essential part of the network of abnormal but not normal cells [121,125,126]. We also find compensatory mechanisms whereby the mutation of one allele encoding a TF leads to shift in the GRN so that it now is dependent on the function of the wild-type allele [127]. Compensatory mechanisms and rewiring of signaling pathways are also common and tend to appear during therapy with the development of different subclonal populations. Examples for this phenomenon are the eradication of cells carrying a mutant FLT3 growth factor receptor after FLT3 inhibitor therapy and the appearance of RAS mutant cells either from preleukemic cells carrying the original driver mutation or from mutated leukemic cell escaping therapy [118,128]. A glimmer of hope comes from studies that profiled the chromatin landscape and gene expression of prospectively isolated subclonal population pairs from different patients carrying different founder mutations. Each subclonal population displayed a different chromatin accessibility pattern indicating that the acquisition of additional genetic changes led to the formation of different chromatin landscapes [129]. However, when different subclonal pairs from different patients were compared, the chromatin accessibility patterns of each pair still clustered in a patient-specific way, demonstrating that epigenetic landscapes cannot drift apart in a disorderly way, that is, the cells have still much in common. Identifying the nature of these commonalities together with the differences will be crucial for the identification of patient-specific therapies.

**Perspectives**

In this review, we have only been able to show a glimpse of the complexity of the gene regulatory mechanisms that are encoded in our genome and that drive cell differentiation and we face significant challenges in our understanding of the molecular basis of developmental processes. We have deliberately left out the RNA world, and we have not mentioned how protein–protein interactions and metabolic processes impact on genome function and many other regulatory processes, many of which also play part in multiple pathologies. For our understanding of cancer as described above, it becomes clear that (a) each type of cancer has to be
seen as a different entity with an entirely unique underlying biology, (b) that we need to understand this biology if we want to get away from therapeutic approaches that target unregulated growth only (chemotherapy) which in itself is genotoxic, and (c) that we need to start thinking how we can reprogram GRNs without touching normal cells. Note, that these statements are valid for a number of pathological processes. We need to directly target the gene regulatory machinery in a disease-specific way, and we need to block the compensatory escape routes that are used by cancer cells, be it the rewiring of signaling pathways or increasing genomic instability thus jumbling GRNs and speeding up evolution. With the development of drugs targeting TFs such as MYC [130], RUNX [131,132], chromatin regulators (BET [133,134], MLL [135]), and repair mechanisms (ATMi [135–137], PARPi [138]), we are starting to develop the right tool box. However, what is clear is that neither our normal environment nor pathological processes can be understood without knowing the rules of gene regulation and cellular biology and the players dictating these rules. This review is a passionate appeal to keep studying how life operates in all its amazing complexity.

Acknowledgements

The research in C. Bonifer’s Lab is funded by grants from the Biotechnology and Biological Sciences Research Council, UK, the Medical Research Council, UK, and Blood Cancer, UK.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

BE-W and CB both contributed to writing the paper.

References

1 Elsaid R, Soares-da-Silva F, Peixoto M, Amiri D, Mackowski N, Pereira P, Bandeira A & Cumano A (2020) Hematopoiesis: a layered organization across chordate species. Front Cell Dev Biol 8, 606642.
2 Ivanovs A, Rybtsv S, Ng ES, Stanley EG, Elefanty AG & Medvinsky A (2017) Human haematopoietic stem cell development: from the embryo to the dish. Development 144, 2323–2337.
3 Palis J, McGrath KE & Kingsley PD (1995) Initiation of hematopoiesis and vasculogenesis in murine yolk sac explants. Blood 86, 156–163.
4 Moore MA & Metcalf D (1970) Ontogeny of the haemopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. Br J Haematol 18, 279–296.
5 Medvinsky A & Dzierzak E (1996) Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 86, 897–906.
6 Pevny L, Simon MC, Robertson E, Klein WH, Tsai SF, D’Agati V, Orkin SH & Costantini F (1991) Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature 349, 257–260.
7 Velten L, Haas SF, Raffel S, Blaszkiewicz S, Islam S, Hennig BP, Hirche C, Lutz C, Buss EC, Nowak D et al. (2017) Human haematopoietic stem cell lineage commitment is a continuous process. Nat Cell Biol 19, 271–281.
8 Liggett LA & Sankaran VG (2020) Unraveling hematopoiesis through the lens of genomics. Cell 182, 1384–1400.
9 Ton MN, Guibentif C & Gottgens B (2020) Single cell genomics and developmental biology: moving beyond the generation of cell type catalogues. Curr Opin Genet Dev 64, 66–71.
10 Bonifer C, Levantini E, Kouskoff V & Lacaud G (2017) Runx1 structure and function in blood cell development. In RUNX Proteins in Development and Cancer (Groner Y, Ito Y, Liu P, Neil JC, Speck NA & van Wijnen A, eds), pp. 65–81, Springer Singapore, Singapore.
11 Growney JD, Shigematsu H, Li Z, Lee BH, Adelsperger J, Rowan R, Curley DP, Kutok JL, Akashi K, Williams IR et al. (2005) Loss of Runx1 perturbs adult hematopoiesis and is associated with a myeloproliferative phenotype. Blood 106, 494–504.
12 Mikkola HK, Klintman J, Yang H, Hock H, Schlaeger TM, Fujiwara Y & Orkin SH (2003) Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. Nature 421, 547–551.
13 Kassouf MT, Hughes JR, Taylor S, McGowan SJ, Soneji S, Green AL, Vyas P & Porcher C (2010) Genome-wide identification of TAL1’s functional targets: insights into its mechanisms of action in primary erythroid cells. Genome Res 20, 1064–1083.
14 Marin M, Karis A, Visser P, Grosveld F & Philipsen S (1997) Transcription factor Sp1 is essential for early embryonic development but dispensable for cell growth and differentiation. Cell 89, 619–628.
15 Gilmour J, O’Connor L, Middleton CP, Keane P, Gillemans N, Cazier JB, Philipsen S & Bonifer C (2019) Robust hematopoietic specification requires the ubiquitous Sp1 and Sp3 transcription factors. Epigenetics Chromatin 12, 33.
Transcriptional control of hematopoiesis

16 Gilmour J, Assi SA, Jaegle U, Kulu D, van de Werken H, Clarke D, Westhead DR, Philipson S & Bonifer C (2014) A crucial role for the ubiquitously expressed transcription factor Sp1 at early stages of hematopoietic specification. Development 141, 2391–2401.

17 Johnson KD, Conn DJ, Shishkova E, Katsumura KR, Liu P, Shen S, Ranheim EA, Kraus SG, Wang W, Calvo KR et al. (2020) Constructing and deconstructing GATA2-regulated cell fate programs to establish developmental trajectories. J Exp Med 217, e20191526.

18 Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, Dafou D, Kilo T, Smithson S, Lunt P et al. (2011) Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet 43, 929–931.

19 Rosenbauer F, Wagner K, Kutock JL, Iwasaki H, Le Beau MM, Okuno Y, Akashi K, Fiering S & Tenen DG (2004) Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. Nat Genet 36, 624–630.

20 Lie ALM, Marinopoulou E, Lilly AJ, Challinor M, Patel R, Lancerin C, Kouskoff V & Lacaud G (2018) Regulation of RUNX1 dosage is crucial for efficient blood formation from hemogenic endothelium. Development 145, dev149419.

21 Lie ALM, Mevel R, Patel R, Blyth K, Baena E, Kouskoff V & Lacaud G (2020) RUNX1 dosage in development and cancer. Mol Cells 43, 126–138.

22 Beck D, Thoms JA, Perera D, Schutte J, Unnikrishnan A, Knezevic K, Kinston SJ, Wilson NK, O’Brien TA, Gottgens B et al. (2013) Genome-wide analysis of transcriptional regulators in human HSPCs reveals a densely interconnected network of coding and noncoding genes. Blood 122, e1–e22.

23 Wilson NK, Foster SD, Wang X, Knezevic K, Schutte J, Kaimakis P, Chlarska PM, Kinston S, Owuhand WH, Dzierzak E et al. (2010) Combinatorial transcriptional control in blood/stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. Cell Stem Cell 7, 532–544.

24 Khourry P, Girardot C, Ciglar L, Peng PC, Gustafson EH, Sinha S & Furlong EE (2017) Uncoupling evolutionary changes in DNA sequence, transcription factor occupancy and enhancer activity. Elife 6, e28440.

25 Obier N, Cauchy P, Assi SA, Gilmour J, Lie ALM, Lichtinger M, Hoogenkamp M, Noailles L, Cockerill PN, Lacaud G et al. (2016) Cooperative binding of AP-1 and TEAD4 modulates the balance between vascular smooth muscle and hemogenic cell fate. Development 143, 4324–4340.

26 Kim WY, Sieweke M, Ogawa E, Wate HI, Englemeier U, Graf T & Ito Y (1999) Mutual activation of Ets-1 and AML1 DNA binding by direct interaction of their autoinhibitory domains. EMBO J 18, 1609–1620.

27 Wadman IA, Osada H, Grutz GG, Agulnick AD, Westphal H, Forster A & Rabbits TH (1997) The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. EMBO J 16, 3145–3157.

28 Zhou Q, Liu M, Xia X, Gong T, Feng J, Liu W, Liu Y, Zhen B, Wang Y, Ding C et al. (2017) A mouse tissue transcription factor atlas. Nat Commun 8, 15089.

29 Pimanda JE, Ottersbach K, Knezevic K, Kinston S, Chan WY, Wilson NK, Landry JR, Wood AD, Kolb-Kokocinski A, Green AR et al. (2007) Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. Proc Natl Acad Sci USA 104, 17692–17697.

30 Lichtinger M, Ingram R, Hannah R, Muller D, Clarke D, Assi SA, Lie ALM, Noailles L, Vijayabaskar MS, Wu M et al. (2012) RUNX1 reshapes the epigenetic landscape at the onset of haematopoiesis. EMBO J 31, 4318–4333.

31 Goode DK, Obier N, Vijayabaskar MS, Lie ALM, Lilly AJ, Hannah R, Lichtinger M, Batta K, Florkowska M, Patel R et al. (2016) Dynamic gene regulatory networks drive hematopoietic specification and differentiation. Dev Cell 36, 572–587.

32 Suter DM, Molina N, Gatfield D, Schneider K, Schibler U & Naef F (2011) Mammalian genes are transcribed with widely different bursting kinetics. Science 332, 472–474.

33 Bartman CR, Hamagami N, Keller CA, Giardine B, Hardison RC, Blobel GA & Raj A (2019) Transcriptional burst initiation and polymerase pause release are key control points of transcriptional regulation. Mol Cell 73, 519–532 e4.

34 Shaulian E & Karin M (2002) AP-1 as a regulator of vascular smooth muscle and hemogenic cell fate. Annu Rev Immunol 10, 532–544.

35 Lin JX & Leonard WJ (2019) Fine-tuning cytokine signaling in hematopoiesis and translational networks construct a balanced immune system. Immunology 139, 1–10.

36 Blank U & Karlsson S (2011) The role of Smad signaling in hematopoiesis and translational hematol. Leukemia 25, 1379–1388.

37 Wu Z & Guan KL (2020) Hippo signaling in embryogenesis and development. Trends Biochem Sci 46, 51–63.

38 Clipstone NA & Crabtree GR (1992) Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 357, 695–697.
40 Yoshimi M, Goyama S, Kawazu M, Nakagawa M, Ichikawa M, Imai Y, Kumano K, Asai T, Mulloy JC, Kraft AS et al. (2012) Multiple phosphorylation sites are important for RUNX1 activity in early hematopoiesis and T-cell differentiation. *Eur J Immunol* 42, 1044–1050.

41 Lundin V, Sugden WW, Theodore LN, Sousa PM, Han A, Chou S, Wrighton PJ, Cox AG, Ingber DE, Goessling W et al. (2020) YAP regulates hematopoietic stem cell formation in response to the biomechanical forces of blood flow. *Dev Cell* 52, 446–460 e5.

42 Johnson BV, Bert AG, Ryan GR, Condina A & Cockerill PN (2004) Granulocyte-macrophage colony-stimulating factor enhancer activation requires cooperation between NFAT and AP-1 elements and is associated with extensive nucleosome reorganization. *Mol Cell Biol* 24, 7914–7930.

43 Brignall R, Cauchy P, Bevington SL, Gorman B, Pisco AO, Bagnall J, Boddington C, Row E, England H, Rich K et al. (2017) Integration of kinase and calcium signaling at the level of chromatin underlies inducible gene activation in T cells. *J Immunol* 199, 2652–2667.

44 Schoenherr CJ & Anderson DJ (1995) The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes. *Science* 267, 1360–1363.

45 Nutt SL, Heavey B, Rolink AG & Busslinger M (1999) Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* 401, 556–562.

46 Tagoh H, Ingram R, Wilson N, Salvagiotto G, Warren AJ, Clarke D, Busslinger M & Bonifer C (2006) The mechanism of repression of the myeloid-specific c-fms gene by Pax5 during B lineage restriction. *EMBO J* 25, 1070–1080.

47 Castano J, Aranda S, Bueno C, Calero-Nieto FJ, Mejia-Ramirez E, Mosquera JL, Blanco E, Wang X, Prieto C, Zabaleta L et al. (2019) GATA2 promotes hematopoietic development and represses cardiac differentiation of human mesoderm. *Stem Cell Reports* 13, 515–529.

48 Stopka T, Amanatullah DF, Papetti M & Skoulitchi AI (2005) PU.1 inhibits the erythroid program by binding to GATA-1 on DNA and creating a repressive chromatin structure. *EMBO J* 24, 3712–3723.

49 Pali CG, Cheng Q, Gillespie MA, Shannon P, Mazurczyk M, Napolitani G, Price ND, Ranish JA, Morrissey E, Higgs DR et al. (2019) Single-cell proteomics reveal that quantitative changes in co-expressed lineage-specific transcription factors determine cell fate. *Cell Stem Cell* 24, 812–820 e5.

50 Mylona A, Theilllet FX, Foster C, Cheng TM, Miralles F, Bates PA, Selenko P & Treisman R (2016) Opposing effects of Elk-1 multisite phosphorylation shape its response to ERK activation. *Science* 354, 233–237.

51 Gottgens B (2015) Regulatory network control of blood stem cells. *Blood* 125, 2614–2620.

52 Rothenberg EV & Gottgens B (2021) How hematopoiesis research became a fertile ground for regulatory network biology as pioneered by Eric Davidson. *Curr Opin Hematol* 28, 1–10.

53 Kucinski I, Wilson NK, Hannah R, Kinston SJ, Cauchy P, Lenaerts A, Grosschedl R & Gottgens B (2020) Interactions between lineage-associated transcription factors govern haematopoietic progenitor states. *EMBO J* 39, e104983.

54 Hesselberth JR, Chen X, Zhang Z, Sabo PJ, Sandstrom R, Reynolds AP, Thurman RE, Neph S, Kuehn MS, Noble WS et al. (2009) Global mapping of protein-DNA interactions in vivo by digital genomic footprinting. *Nat Methods* 6, 283–289.

55 Gilmour J, Assi SA, Noailles L, Lichtinger M, Obier N & Bonifer C (2018) The co-operation of RUNX1 with LDB1, CDK9 and BRD4 drives transcription factor complex relocation during haematopoietic specification. *Sci Rep* 8, 10410.

56 Davidson EH (2010) Emerging properties of animal gene regulatory networks. *Nature* 468, 911–920.

57 Agarwal V & Shendure J (2020) Predicting mRNA abundance directly from genomic sequence using deep convolutional neural networks. *Cell Rep* 31, 107663.

58 Zaret KS (2020) Pioneer transcription factors initiating gene network changes. *Annu Rev Genet* 54, 367–385.

59 Zhu F, Farnung L, Kaasinen E, Sahu B, Yin Y, Wei B, Dodonova SO, Nitta KR, Morgunova E, Taipale M et al. (2018) The interaction landscape between transcription factors and the nucleosome. *Nature* 562, 76–81.

60 Kassabov SR, Zhang B, Persinger J & Bartholomew B (2003) SWI/SNF unwraps, slides, and rewraps the nucleosome. *Mol Cell* 11, 391–403.

61 Voss TC, Schiltz RL, Sung MH, Yen PM, Stamatoyannopoulos JA, Biddie SC, Johnson TA, Miranda TB, John S & Hager GL (2011) Dynamic exchange at regulatory elements during chromatin remodeling underlies assisted loading mechanism. *Cell* 146, 544–554.

62 Goldstein I, Paakinsono V, Baek S, Sung MH & Hager GL (2017) Synergistic gene expression during the acute phase response is characterized by transcription factor assisted loading. *Nat Commun* 8, 1849.

63 Cockerill PN (2011) Structure and function of active chromatin and DNase I hypersensitive sites. *FEBS J* 278, 2182–2210.

64 Xu B, Cai L, Butler JM, Chen D, Lu X, Allison DF, Lu R, Rafii S, Parker JS, Zheng D et al. (2018) The chromatin remodeler BPTF activates a stemness gene-expression program essential for the maintenance of
adult hematopoietic stem cells. Stem Cell Reports 10, 675–683.

65 Hsu J, Huang HT, Lee CT, Choudhuri A, Wilson NK, Abraham BJ, Moignard V, Kucinski I, Yu S, Hyde RK et al. (2020) CHD7 and Runx1 interaction provides a braking mechanism for hematopoietic differentiation. Proc Natl Acad Sci USA 117, 23626–23635.

66 Tu J, Liu X, Jia H, Reilly J, Yu S, Cai C, Liu F, Lv Y, Huang Y, Lu Z et al. (2020) The chromatin remodeler Brg1 is required for formation and maintenance of hematopoietic stem cells. FASEB J 34, 11997–12008.

67 Dekker J, Ripke P, Dekker M & Kleckner N (2002) Capturing chromosome conformation. Science 295, 1306–1311.

68 de Laat W & Grosveld F (2003) Spatial organization of gene expression: the active chromatin hub. Chromosome Res 11, 447–459.

69 Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, Hoke HA & Young RA (2013) Super-enhancers in the control of cell identity and disease. Cell 155, 934–947.

70 Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI & Young RA (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 153, 307–319.

71 Bonifer C, Jagle U & Huber MC (1997) The chicken lysozyme locus as a paradigm for the complex developmental regulation of eukaryotic gene loci. J Biol Chem 272, 26075–26078.

72 Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H & Glass CK (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 38, 576–589.

73 Corces MR, Buenrostro JD, Wu B, Greenside PG, Chan SM, Koenig JL, Snyder MP, Pritchard JK, Kundaje A, Greenleaf WJ et al. (2016) Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. Nat Genet 48, 1193–1203.

74 Fuda NJ, Ardehali MB & Lis JT (2009) Defining mechanisms that regulate RNA polymerase II transcription in vivo. Nature 461, 186–192.

75 Behera V, Stonestrom AJ, Hamagami N, Hsiung CC, Keller CA, Giardine B, Sidoli S, Yuan ZF, Bhanu NV, Werner MT et al. (2019) Interrogating histone acetylation and BRD4 as mitotic bookmarks of transcription. Cell Rep 27, 400–415 e5.

76 Palozola KC, Lerner J & Zaret KS (2019) A changing paradigm of transcriptional memory propagation through mitosis. Nat Rev Mol Cell Biol 20, 55–64.

77 Bevington SL, Keane P, Soley JK, Tauch S, Gajdasiak DW, Fiancette R, Matei-Rascu V, Willis CM, Withers DR & Cockerill PN (2020) IL-2/IL-7-inducible factors pioneer the path to T cell differentiation in advance of lineage-defining factors. EMBO J 39, e105220.

78 Kamada R, Yang W, Zhang Y, Patel MC, Yang Y, Ouda R, Dey A, Wakabayashi Y, Sakaguchi K, Fujita T et al. (2018) Interferon stimulation creates chromatin marks and establishes transcriptional memory. Proc Natl Acad Sci USA 115, E9162–E9171.

79 Bonifer C & Cockerill PN (2017) Chromatin priming of genes in development: concepts, mechanisms and consequences. Exp Hematol 49, 1–8.

80 Cochella L & Hobert O (2012) Embryonic priming of a miRNA locus predetermines postmitotic neuronal left/right asymmetry in C. elegans. Cell 151, 1229–1242.

81 Pijuan-Sala B, Griffiths JA, Guibentif C, Hiscock TW, Jawaid W, Calero-Nieto FJ, Mulas C, Ibarra-Soria X, Tyser RCV, Ho DLL et al. (2019) A single-cell molecular map of mouse gastrulation and early organogenesis. Nature 566, 490–495.

82 Digre A & Lindskog C (2020) The Human Protein Atlas: spatial localization of the human proteome in health and disease. Protein Sci 30, 218–233.

83 Szabo Q, Donjon A, Jerkovic I, Papadopoulos GL, Cheutin T, Bonev B, Nora EP, Bruneau BG, Bantignies F & Cavalli G (2020) Regulation of single-cell genome organization into TADs and chromatin nanodomains. Nat Genet 52, 1151–1157.

84 Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO et al. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326, 289–293.

85 Hildebrand EM & Dekker J (2020) Mechanisms and functions of chromosome compartmentalization. Trends Biochem Sci 45, 385–396.

86 Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS & Zhang Y (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298, 1039–1043.

87 de Napoles M, Mermod JE, Wakao R, Tang YA, Endoh M, Appanah R, Nesterova TB, Silva J, Otte AP, Vidal M et al. (2004) Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. Dev Cell 7, 663–676.

88 Denholtz M, Bonora G, Chronis C, Splinter E, de Laat W, Ernst J, Pellegrini M & Plath K (2013) Long-range chromatin contacts in embryonic stem cells reveal a role for pluripotency factors and polycomb proteins in genome organization. Cell Stem Cell 13, 602–616.

89 Schoenefelder S, Sugar R, Dimond A, Javierre BM, Armstrong H, Mifsud B, Dimitrova E, Matheson L,
Transcriptional control of hematopoiesis

B. Edginton-White and C. Bonifer

Tavares-Cadete F, Furlan-Magaril M et al. (2015) Polycomb repressive complex PRC1 spatially constrains the mouse embryonic stem cell genome. Nat Genet 47, 1179–1186.

90 Boettiger AN, Bintu B, Moffitt JR, Wang S, Beliveau BJ, Fuendenberg G, Imakaev M, Mirny LA, Wu CT & Zhuang X (2016) Super-resolution imaging reveals distinct chromatin folding for different epigenetic states. Nature 529, 418–422.

91 Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W et al. (2008) Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 453, 948–951.

92 Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS & Ren B (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485, 376–380.

93 Nora EP, Lajoie BR, Schulz EG, Gioretti L, Meisig J, Sedat J et al. (2012) Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485, 381–385.

94 Phillips-Cremins JE, Sauria ME, Sanyal A, Gerasimova TI, Lajoie BR, Bell JS, Ong CT, Hoekway TA, Guo C, Sun Y et al. (2013) Architectural protein subclasses shape 3D organization of genomes during lineage commitment. Cell 153, 1281–1295.

95 Nora EP, Goloborodko A, Valton AL, Gibeus JH, Uebersohn A, Abdennur N, Dekker J, Mirny LA & Bruneau BG (2017) Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. Cell 169, 930–944, e22.

96 Kim S & Shendure J (2019) Mechanisms of interplay between transcription factors and the 3D genome. Mol Cell 76, 306–319.

97 Javvierre BM, Burren OS, Wilder SP, Kreuzhuber R, Hill SM, Sewitz S, Cairns J, Wingett SW, Varnai C, Thiecke MJ et al. (2016) Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. Cell 167, 1369–1384 e19.

98 Ganji M, Shaltiel IA, Bisht S, Kim E, Kalichava A, Haering CH & Dekker C (2018) Real-time imaging of DNA loop extrusion by condensin. Science 360, 102–105.

99 Shin Y & Brangwynne CP (2017) Liquid phase condensation in cell physiology and disease. Science 357, eaaf4382.

100 Strom AR & Brangwynne CP (2019) The liquid nucleome – phase transitions in the nucleus at a glance. J Cell Sci 132, jcs235093.

101 Shevtsov SP & Dundr M (2011) Nucleation of nuclear bodies by RNA. Nat Cell Biol 13, 167–173.

102 Nava MM, Miroshnikova YA, Biggs LC, Whitefield DB, Metge F, Boucas J, Vihinen H, Jokitalo E, Li X, García Arcos JM et al. (2020) Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage. Cell 181, 800–817 e22.

103 Larson AG, Elnatan D, Keenen MM, Trnka MJ, Johnston JB, Burlingame AL, Agard DA, Redding S & Narlikar GJ (2017) Liquid droplet formation by HP1alpha suggests a role for phase separation in heterochromatin. Nature 547, 236–240.

104 Uversky VN (2017) Intrinsically disordered proteins in overcrowded milieu: membrane-less organelles, phase separation, and intrinsic disorder. Curr Opin Struct Biol 44, 18–30.

105 Boija A, Klein IA, Sabari BR, Dall’Agnese A, Coffey EL, Zamudio AV, Li CH, Shrinivas K, Manteiga JC, Hannett NM et al. (2018) Transcription factors activate genes through the phase-separation capacity of their activation domains. Cell 175, 1842–1855 e16.

106 Wang Y, Zolotarev N, Yang CY, Rambold A, Mittler G & Grosschedl R (2020) A prion-like domain in transcription factor EBF1 promotes phase separation and enables B cell programming of progenitor chromatin. Immunity 53, 1151–1167 e6.

107 Staby L, O’Shea C, Willemoes M, Theisen F, Kragelund BB & Skriver K (2017) Eukaryotic transcription factors: paradigms of protein intrinsic disorder. Biochem J 474, 2509–2532.

108 Gibson BA, Doolittle PK, Schneider MWG, Jensen LE, Gamarra N, Henry L, Gerlich DW, Redding S & Rosen MK (2019) Organization of chromatin by intrinsic and regulated phase separation. Cell 179, 470–484 e21.

109 Berchtold D, Battich N & Pelkmans L (2018) A systems-level study reveals regulators of membrane-less organelles in human cells. Mol Cell 72, 1035–1049 e5.

110 Jackson DA, Hassan AB, Errington RJ & Cook PR (1993) Visualization of focal sites of transcription within human nuclei. EMBO J 12, 1059–1065.

111 Hnizd D, Shrinivas K, Young RA, Chakraborty AK & Sharp PA (2017) A phase separation model for transcriptional control. Cell 169, 13–23.

112 Shrinivas K, Sabari BR, Coffey EL, Klein IA, Boija A, Zamudio AV, Schuijers J, Hannett NM, Sharp PA, Young RA et al. (2019) Enhancer features that drive formation of transcriptional condensates. Mol Cell 75, 549–561 e7.

113 Guo YE, Manteiga JC, Henninger JE, Sabari BR, Dall’Agnese A, Hannett NM, Spille JH, Afeyan LK, Zamudio AV, Shrinivas K et al. (2019) Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. Nature 572, 543–548.

114 McSwiggen DT, Hansen AS, Teves SS, Marie-Nelly H, Hao Y, Heckert AB, Umemoto KK, Dugast-
Darzacq C, Tjian R & Darzacq X (2019) Evidence for DNA-mediated nuclear compartmentalization distinct from phase separation. Elife 8, e47098.

115 McSwiggen DT, Mir M, Darzacq X & Tjian R (2019) Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. Genes Dev 33, 1619–1634.

116 Churpek JE & Bresnick EH (2019) Transcription factor mutations as a cause of familial myeloid neoplasms. J Clin Invest 129, 476–488.

117 Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, Hoadley K, van Doorn-Khosrovani S, Boer JM, Faucett JA, Gerlinger M, Golub TR, Meltzer PS, Mesirov JP, Rees M, Rosenwald A, Spellman PT, Tamayo P, Ward DF, Zhu X, Shurtleff SA, Botstein D, Treiger B, Mesirov JP, Mesirov JP, Treiger B, Mesirov JP, Mesirov JP, Treiger B, Mesirov JP, Mesirov JP, Treiger B, Mesirov JP, Mesirov JP, Treiger B, Mesirov JP, Mesirov JP, Treiger B, Mesirov JP, Mesirov JP.

118 Miles LA, Bowman RL, Merlinsky TR, Csete IS, Ooi AT, Durruthy-Durruthy R, Bowman M, Famulare C, Patel MA, Mendez P et al. (2020) Single-cell mutation analysis of clonal evolution in myeloid malignancies. Nature 587, 477–482.

119 Morita K, Wang F, Jahn K, Hu T, Tanaka T, Sasaki M, Yasuoka H, Tsunoda T, Yamanaka S, Hamada H, et al. (2015) Chemical biology. A small-molecule inhibitor of CBF alters the chromatin landscape and growth of early human myeloid precursor cells. Cell 162, 1067–1073.

120 Valk PJ, Verhaak RG, Beijen MA, Erpelincik CA, van Waalwijk B, van Doorn-Khosrovan Si, Boer JM, Beverloo HB, Moorhouse MJ, van der Spek PJ et al. (2004) Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 350, 1617–1628.

121 Assi SA, Imperato MR, Coleman DJL, Pickin A, Potluri S, Ptasinska A, Chin PS, Blair H, Cauchy P, James SR et al. (2019) Subtype-specific regulatory network rewiring in acute myeloid leukemia. Nat Genet 51, 151–162.

122 Matheny CJ, Speck ME, Cushing PR, Zhou Y, Corpora T, Regan M, Newman M, Roudaia L, Speck CL, Gu TL et al. (2007) Disease mutations in RUNX1 and RUNX2 create nonfunctional, dominant-negative, or hypomorphic alleles. EMBO J 26, 1163–1175.

123 Kellaway SG, Keane P, Edgington-White B, Regha K, Kennett E & Bonifer C (2021) Different mutant RUNX1 oncoproteins program alternate haematopoietic differentiation trajectories. Life Sci Alliance 4, e202000684. ePub ahead of print.

124 Nafria M, Keane P, Ng ES, Stanley EG, Elefanty AG & Bonifer C (2020) Expression of RUNX1-ETO rapidly alters the chromatin landscape and growth of early human myeloid precursor cells. Cell Rep 31, 107691.

125 Ray D, Kwon SY, Tugoh H, Heidenreich O, Ptasinska A & Bonifer C (2013) Lineage-inappropriate PAX5 expression in t(8;21) acute myeloid leukemia requires signaling-mediated abroga tion of polycomb repression. Blood 122, 759–769.

126 Somerville TD, Wiseman DH, Spencer GJ, Huang X, Lynch JT, Leong HS, Williams EL, Cheesman E & Somervaille TC (2015) Frequent derepression of the mesenchymal transcription factor gene FOXC1 in acute myeloid leukemia. Cancer Cell 28, 329–342.

127 Ben-Ami O, Friedman D, Leshkowitz D, Goldenberg D, Orlovsky K, Pencovich N, Lotem J, Tanay A & Groner Y (2013) Addiction of t(18;21) and inv(16) acute myeloid leukemia to native RUNX1. Cell Rep 4, 1131–1143.

128 McMahon CM, Ferg T, Canaani J, Wang ES, Morriseste JJD, Eastburn DJ, Pellegrino M, Durruthy-Durruthy R, Watt CD, Asthana S et al. (2019) Clonal selection with RAS pathway activation mediates secondary clinical resistance to selective FLT3 inhibition in acute myeloid leukemia. Cancer Discov 9, 1050–1063.

129 de Boer B, Prick J, Pruis MG, Keane P, Imperato MR, Jacques J, Brouwers-Vos AZ, Hogeling SM, Woolthuis CM, Nijk MT et al. (2018) Prospective isolation and characterization of genetically and functionally distinct AML subclones. Cancer Cell 34, 674–689 e8.

130 Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, Sodi NM, Karnezis AN, Swigart LB, Nasi S & Evan GI (2008) Modelling Myc inhibition as a cancer therapy. Nature 455, 679–683.

131 Illendula A, Pulikkkan JA, Zong H, Grembecka J, Xue L, Sen S, Zhou Y, Boulton A, Kuntimaddi A, Gao Y et al. (2015) Chemical biology. A small-molecule inhibitor of the aberrant transcription factor CBFBeta-SMMHC delays leukemia in mice. Science 347, 779–784.

132 Cunningham L, Finckbeiner S, Hyde RK, Southall N, Marugan J, Yadavalli VR, Dehdashi SJ, Reinhold WC, Alemu L, Zhao L et al. (2012) Identification of benzodiazepine Ro5-3335 as an inhibitor of CBF leukemia through quantitative high throughput screen against RUNX1-CBFBeta interaction. Proc Natl Acad Sci USA 109, 14592–14597.

133 Filipiakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I et al. (2010) Selective inhibition of BET bromodomains. Nature 468, 1067–1073.

134 Kritsvov AV, Evans K, Gadrey JY, Eschle BK, Hatton C, Uckermann HJ, Ross KN, Perner F, Olsen SN, Pritchard T et al. (2019) A Menin-MLL inhibitor induces specific chromatin changes and eradicates disease in models of MLL-rearranged leukemia. Cancer Cell 36, 660–673 e11.

135 Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AJ, Reaper PM, Jackson SP, Curtin NJ & Smith GC (2004) Identification and characterization of a novel and specific inhibitor of the ataxia telangiectasia mutated kinase ATM. Cancer Res 64, 9152–9159.
136 Pike KG, Barlaam B, Cadogan E, Campbell A, Chen Y, Colclough N, Davies NL, de-Almeida C, Degorce SL, Didelot M et al. (2018) The identification of potent, selective, and orally available inhibitors of ataxia telangiectasia mutated (ATM) kinase: the discovery of AZD0156 (8-[6-[3-(Dimethylamino)propoxy]pyridin-3-yl]-3-methyl-1-(tetrahydro-2 H-pyran-4-yl)-1,3-dihydro-2 H-imidazo[4,5- c]quinolin-2-one). J Med Chem 61, 3823–3841.

137 Karlin J, Allen J, Ahmad SF, Hughes G, Sheridan V, Oedera R, Farrington P, Cadogan EB, Riches LC, Garcia-Trinidad A et al. (2018) Orally bioavailable and blood-brain barrier-penetrating ATM inhibitor (AZ32) radiosensitizes intracranial gliomas in mice. Mol Cancer Ther 17, 1637–1647.

138 Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshow JH, Ji J, Takeda S & Pommier Y (2012) Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res 72, 5588–5599.