Advances in targeting 'undruggable' transcription factors with small molecules

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Abstract | Transcription factors (TFs) represent key biological players in diseases including cancer, autoimmunity, diabetes and cardiovascular disease. However, outside nuclear receptors, TFs have traditionally been considered 'undruggable' by small-molecule ligands due to significant structural disorder and lack of defined small-molecule binding pockets. Renewed interest in the field has been ignited by significant progress in chemical biology approaches to ligand discovery and optimization, especially the advent of targeted protein degradation approaches, along with increasing appreciation of the critical role a limited number of collaborators play in the regulation of key TF effector genes. Here, we review current understanding of TF-mediated gene regulation, discuss successful targeting strategies and highlight ongoing challenges and emerging approaches to address them.

DNA-binding transcription factors (TFs) represent one of the most essential classes of proteins in the eukaryotic proteome[1]. By binding to specific DNA sites and controlling transcriptional output of genes in close spatial proximity, TFs play foundational roles in the regulation of virtually all of a cell's genome[2]. TFs dictate the identity and fate of individual cells in multicellular organisms by differentially regulating the common genetic code, and are responsible for rapidly coordinating responses to internal and external stimuli by serving as end points in cell signalling networks[3,4]. It is estimated that there are at least 1,600 TFs in the human genome, around 19% of which have been associated with a disease phenotype[1]. Concordantly, given their central importance to biology, TFs are frequent drivers of disease and represent tantalizing therapeutic targets[3,5,6].

The significant potential of direct TF modulators was best encapsulated almost two decades ago by James Darnell in the context of anticancer therapeutics[5]. He highlighted how TFs, more so than upstream signalling proteins such as GPCRs or kinases, have the capacity for highly specific disease modulation given their foundational role in selective gene regulation. That is, a hypothetical inhibitor of a dysregulated TF could limit toxicity while increasing efficacy by only inhibiting transcriptional programmes driven by that TF, without the collateral damage sometimes associated with inhibiting signalling proteins that are involved with multiple signalling networks unrelated to the disease[7,8]. Because individual TFs typically only regulate a limited set of gene targets that are governed by their DNA-binding specificity, such an inhibitor is also likely to be less prone to compensatory resistance mechanisms common to other pharmacological modalities such as tyrosine kinase inhibitors[9]. This exceptional potential of therapeutically modulating TF action is illustrated by the enduring success of myriad nuclear receptor-targeting drugs, which represent the standard of care across several different disease areas[10].

Despite the broad therapeutic promise of TF modulators, there are major roadblocks associated with TFs as a target class that have impeded countless attempts at drugging TFs outside the nuclear receptor family. Consequently, of the roughly 300 TFs that have been associated with a disease phenotype, only a handful have been successfully targeted by small molecules[1]. A fundamental challenge is that most TFs are predominantly intrinsically disordered and lack classical well-formed small-molecule binding pockets[11]. TFs function primarily by forming highly dynamic protein–DNA interactions and protein–protein interactions (PPIs), and consequently the most critical functional sites also represent exceptionally challenging regions to directly target with small molecules. Beyond just the basic difficulties of TF ligand development, the regulation and function of individual TF domains is often highly complex or poorly understood, obfuscating the domains that would actually be fruitful to modulate. This, combined with continually emerging evidence that challenges our fundamental understanding of gene regulation and TF mechanisms of action[12,13], makes TFs some of the thorniest targets in the proteome.

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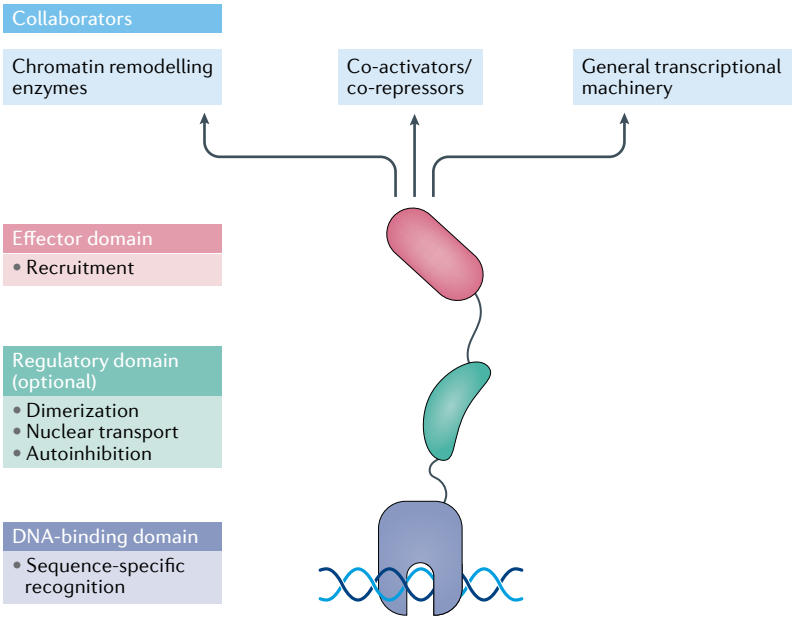


Fig. 1 | **Anatomy of a TF.** All transcription factors (TFs) contain two general protein domains: a DNA-binding domain (DBD) that binds to specific DNA regulatory sequences, and an effector domain that recruits various transcriptional 'collaborators' to regulate chromatin accessibility and transcriptional output. Many TFs also contain one or more regulatory domains, which typically serve to regulate TF localization and functional activity.

This Review synthesizes current understanding of TF function and gene regulation with emerging pharmacological approaches that can or could be used to drug this target class. We discuss the basic mechanisms by which TFs participate in gene regulation and drive myriad diseases, and then evaluate key lessons from successful and promising examples of TF modulator development. We close by highlighting technologies that could facilitate progress in drugging even the most recalcitrant TFs and reflect on how emerging medicinal chemistry, biophysics and chemical biology approaches could be adapted to address the unique challenges associated with TFs.

Functional domains of TFs

The key role of a TF is to recruit transcriptional regulatory machinery to specific genomic loci to regulate gene expression[14]. A minimal TF is thus defined by just the presence of two key elements: a DNA-binding domain (DBD) that recognizes specific DNA sequences, and an effector domain that recruits members of transcriptional activation or repression machinery[14] (FIG. 1). TFs that act as transcriptional activators use a transactivation domain to recruit chromatin remodelling enzymes, histone modifying enzymes, transcriptional co-activators and/or many general TFs to increase the accessibility of target genes, epigenetically mark them as active, and recruit and activate RNA polymerase II (Pol II)[12,14–18]. Conversely, TFs that behave as transcriptional repressors use a transrepression domain to recruit chromatin remodelling and epigenetic enzymes to decrease the accessibility of target genes and mark them as inactive[17]. In some cases, prototypical transactivation domains can have repressive functions that are controlled by the presence and/or activity of co-repressors[19]. By this basic mechanism of recruitment, TFs act as the directors of transcriptional output of the genome and play key roles across wide-ranging cellular processes[18].

The structural and biophysical mechanisms by which key TF regulatory domains function have been a subject of intense study for decades. The determinants of DBD specificity for DNA sequence in vitro have been extensively dissected with advances in high-throughput binding assays and determination of numerous DBD structures, in both the presence and absence of DNA[20]. However, significant challenges in predicting functional TF binding sites in the genome remain, which is complicated by the complex three-dimensional chromatin architecture and an abundance of non-specific TF binding sites that can compete with TF binding to scarcer specific TF binding sites[21].

Conversely, the basic mechanisms by which effector domains function are much less defined. Although there are certainly some instances of well-studied and functionally important PPIs made by single effector domains[22–24], the generalizability of these examples to the class as a whole has not been possible[25–27]. For example, although several structures of transactivation domains bound to co-activators such as CBP/p300 (REF.[24]) have been proposed, these structures do not explain the repeated observation that roughly 1% of any random sequence of amino acids — with the only commonality being the preponderance of acidic and hydrophobic residues — stitched to a DBD can function as transactivation domains[25,27]. Thus, the general mechanisms by which effector domains actually effectuate recruitment are still under considerable debate. Current universal models of effector domain function hypothesize that they form non-specific dynamic PPIs with transcriptional machinery as well as phase-separating with disordered regions of co-activators/co-repressors to form transcriptional condensates[13,28–30], although in some individual cases there is evidence that other mechanisms are more consistent with experimental data[31,32]. Put together, there are many remaining questions about the mechanisms by which the two key TF domains function that may have drastic implications for the success of various targeting strategies.

As well as the two class-defining TF functional domains, many TFs contain additional layers of regulation that add further complexity to their function and regulation (FIG. 1). For example, the STAT family of TFs contain a SH2 domain that controls homodimerization or heterodimerization with other STAT TFs, and thereby regulates the TF localization to the nucleus[33]. Nuclear receptors, by far the most druggable family of TFs, contain a ligand-binding domain (LBD) that typically acts in cooperation with a prototypical disordered transactivation domain to recruit transcriptional machinery when bound to a small-molecule ligand[34]. Other TFs such as the basic helix–loop–helix family require dimerization with other family members to form competent DBDs[35]. These diverse regulatory domains and mechanisms have historically served as the most promising entry points for medicinal chemists to develop effective TF modulators[6].

Non-specific TF binding sites
Sequences of DNA that do not contain the consensus sequence for a transcription factor (TF) DNA-binding domain (DBD). Most DBDs have low affinity for non-specific sites, but because of the exceptionally high ratio of non-specific to specific sites, TFs often spend significant time at non-specific sites.

Specific TF binding sites
Sequences of DNA that contain the consensus sequence for a transcription factor (TF) DNA-binding domain.

Transcriptional condensates
Liquid–liquid phase-separated droplets containing transcription factors, co-activators, RNA polymerase II (Pol II) and other transcriptional machinery.

Pre-initiation complex
A large complex comprising general transcription factors, Mediator and other proteins that position and activate RNA polymerase II (Pol II) at the transcription start site.

Gene regulation by TFs

A key lesson emerging over recent years is that eukaryotic gene regulation is an exceptionally complex and dynamic process that is often counter-intuitive and facilitates many surprising behaviours[12,30,36,37]. Whether a TF functions at a specific binding site depends not only on the thermodynamic stability of the TF–DNA complex but on a number of interoperating factors, including multidimensional DNA/chromatin architecture, the cooperative action of other TFs and co-activators at nearby or overlapping sites and the kinetics of the TF binding to DNA itself[13,21,38–40]. Here, we focus on recent insights into the mechanisms that regulate the strength of TF-driven transcriptional activation.

Influence of genome structure on TF action. For decades, it has been understood that the organization of the genome, across several dimensions, is a key determinant of whether a gene is turned on or off[2]. TFs control the expression of most genes by binding to promoter and/or enhancer regions of DNA[18]. Promoters are characterized by their inclusion of a transcription start site (TSS) and a TATA-box/Inr DNA sequence, the latter of which enables assembly of the pre-initiation complex and subsequent activation of RNA Pol II[2]. Enhancers, conversely, do not contain a functional TSS and can be located up to several kilobases away from a TSS. Enhancers instead bind to TFs and activate transcription when placed in close spatial proximity to a promoter[41]. This reliance on three-dimensional proximity for enhancer function has many remarkable consequences, chiefly that many enhancers only function at genes located at long genomic distances (>1 kb) instead of at closer genomic loci[2]. Although mechanisms of transcriptional activation at promoters have been extensively characterized — down to the structures of the pre-initiation complex at different steps of the activation process[42–44] — understanding how genes are regulated by enhancer regions is still an area of intense study and emerging therapeutically relevant insights.

A breakthrough in understanding how enhancers are placed into proximity of the target genes has been the identification of chromatin neighbourhoods or topologically associated domains (TADs)[38,45–47]. TADs are essentially extruded chromatin loops that are bound by the proteins cohesin and CTCF, and enable cells to dictate the three-dimensional structure of specific regions of the genome[38] (FIG. 2a). TADs are frequently conserved within cell types and are thought to place key cell-identity genes under the control of multiple enhancers to maintain robust expression[48]. Accordingly, TADs can be restructured upon differentiation of progenitor cells as a mechanism to remodel cell-identity transcriptional programmes[46]. Remarkably, not all genes within a TAD are necessarily dependent on the TAD for function, suggesting additional complexities to genomic structure that could be relevant for selective therapeutic targeting of genes within TADs[49].

A particularly noteworthy advance in the basic biology of gene regulation has been the discovery and characterization of super-enhancers[50,51] (FIG. 2a). Super-enhancers are defined as extended clusters of enhancers with particularly elevated levels of bound TFs and co-activators as well as epigenetic marks associated with active transcription (for example, H3K27Ac). Due to their high sustained levels of transcriptional activity, super-enhancers often act in concert with TADs to control expression of key cell-identity genes[48,50,51]. For example, in development, super-enhancers have been observed as regulators of core regulatory TFs that control the process and timeline of cell differentiation[52]. Super-enhancers are also inactivated or repurposed over the course of development to initiate changes in cell characteristics; in cancer, these mechanisms enable malignancies to use super-enhancers to drive oncogenic transcriptional programmes[50,51,53].

Dynamics of TF action. A crucial fact that underlies our current understanding of eukaryotic transcriptional regulation is that it is a highly dynamic and

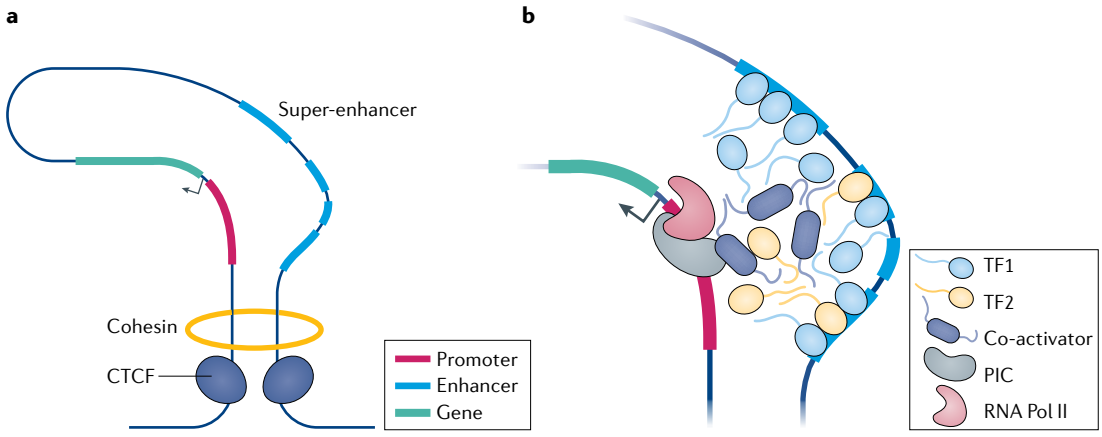


Fig. 2 | **Overview of the modern model of the transcriptional activation process. a** | Depiction of a topologically associated domain (TAD) bound by cohesin and CTCF containing a super-enhancer that controls a gene. **b** | Zoomed view of the phase-separation model of transcriptional activation, where transcription factors (TFs) and co-activators form transcriptional condensates spanning the enhancer and promoter. PIC, pre-initiation complex; RNA Pol II, RNA polymerase II.

out-of-equilibrium process[12]. This has led to several surprising revelations about in vivo mechanisms of TF and co-regulator function that interact intimately with emerging insights about genome structure.

The remarkable dynamics of TF binding to target sites in vivo has several surprising consequences. The classical view of TF–DNA interactions is that TFs reside on DNA for long periods of time to carry out their function, but modern in vivo imaging studies have estimated that the lifetimes of TFs with target DNA sites can be as short as a few seconds[12,40,54,55]. This dynamism is thought to serve as a regulatory mechanism to keep TFs from being trapped at non-specific DNA sites for extended time periods. For example, the relative fraction of binding to specific sites over non-specific sites of some nuclear receptors only marginally increases when activated, but fast turnover and extended lifetimes at specific sites facilitate a rapid and significant increase in transcriptional output at target genes[40].

Dynamic TF–DNA binding can also lead to surprising modes of TF cooperativity. For example, whereas cooperative activity of multiple TFs at a promoter or enhancer is classically thought to be enacted by different TFs binding to adjacent sites and stabilizing each other's binding, rapid TF binding and unbinding has also been found to lead to cooperativity from different TFs binding the same site[12,36]. The low lifetimes and long periods of time between binding of each TF enable unimpeded exchange between different TFs, acting to keep chromatin in an open conformation and to recruit distinct members of the transcriptional apparatus.

Other key examples of unexpected behaviour in transcription have been observed during characterization of super-enhancer-driven transcriptional activation. Strikingly, super-enhancers display marked increases in both the inter-reliance and the binding and unbinding dynamics of TFs and co-activators over typical enhancers[51,56]. The amplified cooperativity at super-enhancers causes them to be especially sensitive to slight changes in TF and co-regulator composition. This can result, for example, in inhibitors of general co-activators such as BRD4 displaying exquisite selectivity for super-enhancer-driven transcription[56]. Inhibitors of general transcriptional regulatory enzymes have even been observed to copy the phenotypes of removing core regulatory TFs in some cell types[57–59]. On the other hand, the action of transcriptional co-repressors at super-enhancers can be paradoxically critical for maintaining maximum transcriptional output, exposing a highly dynamic steady state of chromatin accessibility and TF/co-regulator binding needed for super-enhancer activity[60–62].

Liquid–liquid phase separation (LLPS) has emerged as a popular biophysical framework to rationalize the exceptionally cooperative and dynamic behaviour of TFs and co-regulators at super-enhancers[13,30,37] (FIG. 2b). Significant levels of TF binding at super-enhancers is hypothesized to create high local concentrations of co-activators and other members of the transcriptional machinery; at a critical concentration, these TFs and other cofactors form phase-separated 'transcriptional condensates' spanning the super-enhancer and the TSS[13,30]. Consistent with this notion, multiple experimental efforts have demonstrated that TFs and co-activators form highly concentrated puncta at active super-enhancer sites in vivo[13,30,37,63]. Corresponding in vitro experiments using purified TFs and co-activators have shown that low-complexity intrinsically disordered regions (IDRs) in TFs, co-activators and general transcriptional machinery (including RNA Pol II) have the capacity to co-condense into phase-separated droplets[13,37,64,65].

To many, LLPS serves as an exceedingly useful framework to rationalize otherwise puzzling transcriptional phenomena. For example, IDRs are highly enriched in TFs and co-regulators, but because these regions are disregarded in standard structure–function paradigms, it was previously challenging to understand how they could participate in transcriptional regulation. Within an LLPS framework, it is theoretically possible to identify functions and mechanisms of TF IDRs by simply considering their physicochemical properties, concentration and the landscape of DNA-binding sites at a given enhancer, which together dictate their ability to participate in transcriptional condensates[66]. Transitions into and out of a condensate by a single protein can, consequently, be facilitated by post-translational modifications that change IDR properties[64], and the formation and dissolution of individual condensates can be regulated by fluctuations in composition of the proteins and RNA that are active during the process of transcription[65]. Examples where applications of LLPS frameworks to the dissection of IDR functional mechanisms have given potential answers to otherwise perplexing experimental observations include rationalizations of transcriptional bursting[65], RNA Pol II promoter release[64], enhancer–promoter contact restrictions[67] and the extraordinary sequence diversity of functional transactivation domains[13]. Phase transitions have also been implicated in distinct mechanisms of gene regulation outside transcriptional activation, and are thought to play roles in the function of splicing condensates[64,68], repressive Polycomb repressor complex (PRC) bodies[69] and heterochromatin/euchromatin transitions[70,71].

It is also worth noting that the existence of transcriptional condensates is still somewhat contentious, due to the challenge associated with unequivocally demonstrating that puncta containing TFs and transcriptional machinery in vivo indeed constitute a separated liquid phase[72,73]. Specifically, there have been concerns raised that much of the experimental data are phenomenological, and that other mechanisms could underlie the same observations[32]. Moreover, the difficulty of studying transcriptional condensates is higher than for other well-characterized examples of LLPS due to the highly dynamic and localized nature of transcriptional activation. Nonetheless, there is broad agreement that the formation of dynamic concentrated hubs of TFs and transcriptional apparatus plays a critical role in transcription, especially at super-enhancers[30,37].

Altogether, the often-surprising outcomes of the three-dimensional and dynamic nature of transcription strongly indicate that many general assumptions

Cooperativity
In transcription, a phenomenon where binding of one transcription factor and/or co-regulator at a regulatory element enhances the binding of other factors, and vice versa.

Core regulatory TFs
(Also known as master TFs). Self-regulated transcription factors (TFs) that control cell identity and fate.

about TF action developed from decades of mechanistic studies in simplified systems have significant potential to be misleading[12]. Concordantly, TF mechanism of action may be highly variable from cell type to cell type, gene to gene and binding site to binding site. When selecting possible targets to affect the function of a given TF, including individual domains of the TF itself or its co-regulatory binding partners, unbiased functional data are therefore critical for effective decision-making.

Dysregulated transcription in disease

A principal reason why TFs are considered highly alluring therapeutic targets is that transcriptional dysregulation plays an essential role across a wide variety of diseases[3] (TABLE 1). As the fundamental drivers of selective gene expression, TFs are intimately involved in the dysregulated transcriptional programmes that are key to pathogenesis and, thus, represent some of the most direct targets for disrupting disease[5]. In this section, we highlight disease areas where TFs are important, while discussing common mechanisms of transcriptional dysregulation and the roles that TFs play in this process (FIG. 3).

Cancer. Dysregulated transcription is a hallmark of cancer, and TFs frequently serve as fundamental drivers of oncogenic transformation, proliferation and survival[74]. TFs can be responsible for causing oncogenic phenotypes by a range of diverse mechanisms. Overactivation and/or overexpression of TFs that control growth pathways often drives cancer proliferation[75–77]. Conversely, aberrant inactivation of tumour suppressor TFs enables evasion of apoptosis and cancer survival[78,79].

Table 1 | **Selected examples of TFs that drive disease**

TF	Associated diseases	Dysregulation mechanisms	Refs
Cancer			
MYC	Various forms of cancer	Amplifies oncogenic transcriptional programmes	89,90
MYB	Various forms of cancer	Overactivation by gene duplication, overexpression and genetic fusions to other proteins	84
E2F	Various forms of cancer	Overactivation by dysregulation of co-repressor pRB	19,287
TAL1	T cell acute lymphoblastic leukaemia	Overexpression and overactivation	288
PAX3-FOXO1	Alveolar rhabdomyosarcoma	Oncogenic fusion TF, dysregulates muscle development transcriptional programmes	95,289
p53	Various forms of cancer	Downregulation by the ubiquitin–proteasome system or loss-of-function mutations	141,142
Autoimmune and inflammatory disease			
STAT1	Atherosclerosis, infection	Overactivation by signalling pathways	290
STAT3	Various forms of autoimmune and inflammatory disease, as well as cancer and diabetes	Gain-of-function mutations and/or overactivation by signalling pathways (cancer, autoimmune disease), or loss-of-function mutations (hyper IgE syndrome)	101,102,104,114
STAT6	Asthma and allergy	Overactivation by signalling pathways	103
T-bet	Multiple sclerosis, systematic lupus erythematosus	T_H1 cell master TF, drives and/or increases severity of autoimmunity	106
GATA3	Atopic asthma, allergies	T_H2 cell master TF, drives and/or increases severity of autoimmunity	107,111
RORγt	Psoriasis	T_H17 cell master TF, drives and/or increases severity of autoimmunity	109
FOXP3	IPEX	Loss-of-function mutation	113
NF-κB	Various forms of autoimmune and inflammatory disease, cancer	Overactivation by signalling pathways	77,98,99
Diabetes			
HNF1α	Maturity-onset diabetes of the young	Loss-of-function mutation	117
HNF4α	Maturity-onset diabetes of the young	Loss-of-function mutation	117
NEUROD1	Maturity-onset diabetes of the young	Loss-of-function mutation	117
Cardiovascular disease			
GATA4	Maladaptive cardiac hypertrophy, congenital heart disease	Overactivation (cardiac hypertrophy) or loss-of-function mutation (congenital heart disease)	120,122
Nkx2-5	Congenital heart disease	Loss-of-function mutation	122
Tbx5	Congenital heart disease	Loss-of-function mutation	122

TF, transcription factor; T_H1 cell, T helper 1 cell; T_H2 cell, T helper 2 cell; T_H17 cell, T helper 17 cell.

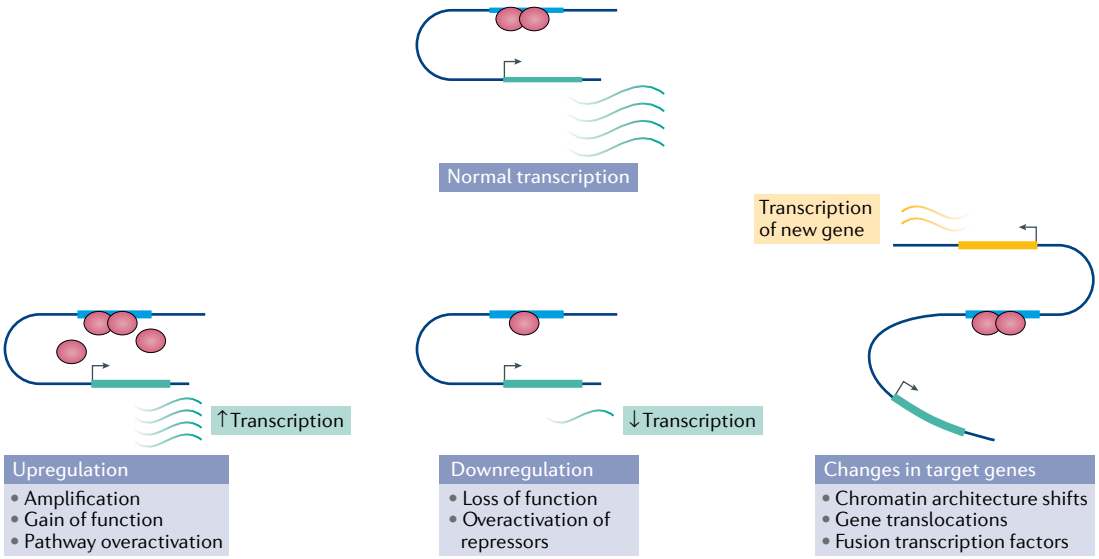


Fig. 3 | **Common mechanisms of transcriptional dysregulation in disease.** Various mechanisms by which the normal transcriptional profile of an example transcription factor (TF) effector gene (top middle) can be dysregulated in disease. Effector genes that drive a disease state can be upregulated by overactive and/or overabundant TFs, which commonly occurs by gene amplification, TF gain-of-function mutations and/or signalling pathway overactivation (bottom left). Effector genes that protect against disease can be aberrantly downregulated by TF loss-of-function mutations and overactivation of repressor proteins of the TF (bottom middle). Genes that are regulated by a TF in normal conditions can change due to chromatin architecture shifts, genetic translocation of enhancers to new effector genes and TF fusions that change or disrupt the DNA specificity of the parent TF(s) (bottom right).

Genetic fusion events that generate fusion TFs are a common cause of paediatric cancers, and typically dysregulate developmental transcriptional programmes to initiate transformation and drive proliferation[80]. Oncogenic viruses are known to initiate transformation via a combination of the activity of viral TFs in addition to other viral proteins that co-opt or dysregulate cellular TFs and transcriptional co-regulators[81]. There are also some cases, for example in certain gliomas[82], where TFs drive oncogenic phenotypes simply by rewiring their transcriptional programmes to regulate a different set of effector genes[83].

The TF MYB serves as an excellent example of a single oncogenic TF that can act by several of the mechanisms outlined above[84]. MYB is intimately involved in a variety of cancers including leukaemia (especially acute myeloid leukaemia (AML)), adenoid cystic carcinoma, colorectal cancer and breast cancer, where it generally drives oncogenesis by becoming overactivated. Most commonly, gene duplications and overexpression of MYB lead to overactivation of MYB target genes, but MYB can also become overactivated by genetic translocations (for example, MYB-NFIB) that fuse it to other proteins, typically eliminating the MYB transrepression domain in the process[83]. In other cases, genetic changes can generate new MYB binding sites that enhance other oncogenic drivers. For example, in some forms of T cell acute lymphoblastic leukaemias, novel MYB binding sites can form in the enhancer for the driver TF TAL1 and increase its expression[85].

The central role of TFs in driving oncogenesis frequently leads to reliance of malignancies on the activity of individual TFs[74]. A classic example of a TF that exhibits this 'oncogenic addiction' behaviour across a variety of cancers is MYC. MYC is a TF in the basic helix–loop–helix family that, along with its binding partner MAX, binds to the widespread E-box sequences at promoters and enhancers across the genome[86]. MYC primarily functions by recruiting transcriptional elongation machinery to enhancers to increase transcriptional output[87,88]. MYC is one of the most frequently overexpressed oncogenes and is thought to act as a general transcriptional 'amplifier' to drive a wide variety of oncogenic transcriptional programmes across diverse cancer types[3,89,90]. In vivo experiments using genetic knockdown of MYC and expression of dominant negative MYC variants have shown that several distinct cancers are addicted to MYC's amplification activity, rapidly dying or differentiating into normal cell types upon MYC inhibition[91–93]. Similarly, TFs as a class represent a large fraction of hits in cancer genetic dependency databases such as DepMap[94], supporting the idea that oncogenic addiction to TFs is a shared vulnerability across myriad cancers. Thus, there is exceptionally high potential for targeting TF activity as a therapeutic strategy for cancer.

In transcription, TFs do not function alone: the fundamental role of TFs is to recruit the requisite machinery to do the work required for transcriptional regulation[14]. Accordingly, much of the apparatus that facilitates TF-driven activation/repression can also be critical for maintaining oncogenic transcriptional programmes. This is especially relevant when considering the important roles that super-enhancers have been shown to play in cancer, given the heightened levels of cooperativity between TFs and cofactors at

Chromatin readers
Proteins, such as bromodomains, that bind to post-translationally modified histones.

these regulatory elements. As discussed previously, in normal cells, super-enhancers often form around key cell-identity genes[50,51,53]; it has similarly been observed that malignancies frequently generate or repurpose super-enhancers around key oncogenic identity and effector genes[53,95]. Super-enhancers thereby grant cancer cells increased and sustained activation of these genes, and are consequently key to maintaining an undifferentiated cell state and enabling rapid and continuous growth.

Because of the exceptionally cooperative nature of super-enhancer function, super-enhancers are frequently dependent on the action of select members of the transcriptional apparatus (for example, chromatin readers, histone modifying enzymes, transcriptional kinases) in addition to TFs[56–60,95,96]. Without even one of these co-regulators and/or its associated enzymatic activity, super-enhancers can be rapidly depleted of TFs, active chromatin marks and the transcriptional apparatus. Unique super-enhancers can also have distinct cofactor dependency profiles, which can enable selective inhibition of super-enhancer-driven oncogenic transcription[57,59]. For example, inhibitors of general transcriptional enzymes such as CDK9 have been shown to display strikingly selective inhibition of the oncogenic transcription programmes of androgen receptor ΔLBD splice variants by this mechanism[59]. Inhibiting the activity of TF collaborators at super-enhancers therefore has significant therapeutic potential for treating cancer and serves as an alternative to targeting the oncogenic TF itself, especially in cases where the TF proves recalcitrant to small-molecule discovery efforts.

Autoimmune/inflammatory disease. TFs are common end points of signalling pathways that mediate the immune response to infection or injury[76,77]. Consequently, dysregulation of TFs involved in immune response plays a significant role in the pathogenesis of autoimmune and inflammatory diseases. For example, the TF NF-κB is a master regulator of both innate and adaptive immunity: among other functions, it controls both the expression of pro-inflammatory cytokines in macrophages as well as the activation and differentiation of naive $CD4^+$ T helper cells[77]. Several diverse signalling pathways regulate activation of NF-κB and its transit to the nucleus. Overactivation of NF-κB activity is strongly linked to myriad inflammatory and/or autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis[77,97–100].

Directly downstream of NF-κB in mediating immune response lies the STAT family of TFs, which regulate the expression of cytokine-inducible genes such as interferons[76]. Individual STAT family members are similarly implicated in numerous autoimmune and inflammatory diseases. Overactivation of STAT activity is, in general, linked to autoimmune disease, for example activating mutations of family member STAT3 have been linked to early-onset type 1 diabetes, Crohn's disease, psoriasis and multiple sclerosis[101,102]. Similarly, overactivation of STAT6 is known to play significant roles in allergy and asthma[103]. Inactivation of STAT family members, on the other hand, often leads to immunodeficiency and predisposition to various types of infection[76]. For example, inactivating mutations in STAT3 underlie many cases of hyper IgE syndrome[104].

T cells are intimately involved in the development, progression and severity of myriad autoimmune diseases[105]. Although the molecular mechanisms by which individual T cell types affect autoimmunity are quite complex, the master TFs that define T cell identity are thought to serve as general orchestrators of many autoimmune diseases. Overactivation/overexpression of master TFs in T helper 1 (T_H1) cells (T-bet), T_H2 cells (GATA3) and T_H17 cells (RORγt), for example, is linked to several T cell-driven autoimmune diseases such as multiple sclerosis, systematic lupus erythematosus, atopic asthma and psoriasis[106–110]. T cell master TFs even, in some cases, protect against autoimmune disease; for example, overexpression of the master TF GATA3 (T_H2 cells) displayed reduced symptom severity of multiple sclerosis in murine models[111]. Similarly, the master TF FOXP3 of regulatory T cells is known to drive the immunosuppressive effects of regulatory T cells and its expression is correlated to decreased severity of autoimmune disease[112]. Inactivation of FOXP3, on the other hand, is highly deleterious and can lead to X-linked congenital immunodeficiency syndromes[113].

Altogether, immune response and T cell master regulatory TFs make enticing targets for the numerous diseases caused by aberrant immune responses, especially given their rich regulatory networks that provide several possible intervention points[76,77,114]. We also note that many of these TFs often have a direct relationship to cancer as well, where dysregulated immune response TFs have been shown to play critical roles in enabling transformation, invasion and metastasis[33,77].

Diabetes. Diabetes mellitus, characterized by an inability to properly secrete or utilize insulin, is, in general, a polygenic disease linked to changes in several genes simultaneously[115]. However, there are some forms of monogenic diabetes that have been directly linked to mutations in single TFs. For example, as previously mentioned, activating STAT3 mutations have been linked to early-onset type 1 diabetes[101]. A significant form of monogenic diabetes is maturity-onset diabetes of the young (MODY), which accounts for around 2% of all diabetes cases in patients younger than 20 years old[116]. Five of the six genes that have been directly linked to MODY are TFs, which include the hepatic nuclear factors HNF1α, HNF1β and HNF4α, the insulin promoter factor IPF1 and NEUROD1 (REF.[117]). In all cases, loss-of-function mutations lead to MODY. Interestingly, the vast majority of MODY cases are caused by mutations in one of the three hepatic nuclear factors that are primarily associated with liver function and previously had no obvious connection to β-cells[117]. Conversely, only a small fraction of MODY cases are caused by mutations in IPF1 or NEUROD1, even though they both directly regulate insulin expression. HNF1α, HNF1β and HNF4α are also known to cooperate directly to regulate target gene expression[118], and thus possible therapeutic strategies may include the development of agonists against one of these TFs to restore overall function.

Cardiovascular disease. Cardiovascular disease, like diabetes, is a group of diseases that are linked to multiple interrelated genetic risk factors and are not necessarily driven by single proteins. However, TFs are critical to the development and maintenance of the cardiovascular system, and thus can play significant roles in certain forms of cardiovascular disease[119,120]. Congenital heart defects, for example, are commonly linked to loss-of-function mutations in master TFs that control development of the cardiovascular system[119,121,122]. Core regulatory cardiovascular TFs such as GATA, HAND, MEF2 and SRF also play critical roles in directing the response of the cardiovascular system to stress, and overactivation of these TFs to stimuli such as pressure and volume overload can lead to maladaptive cardiac hypertrophy[120]. Therapeutic modulation of key cardiovascular TFs therefore has the potential for treating several forms of cardiovascular disease.

Advances in targeting TFs

Modulation of TFs by small molecules is an alluring therapeutic objective given their importance across numerous diseases[3,5]. However, outside nuclear hormone receptors[10], few drugs or even well-validated chemical probes are known to directly target TFs. Further, many consider TFs to be predominantly 'undruggable' because they have significant structural disorder and lack classical small-molecule binding pockets[5,6]. The basic mechanisms by which TFs function also contribute to this image: most known effector domains are disordered when unbound to partner proteins[11], and whereas DBDs are typically more structured, DNA-binding surfaces tend to be highly charged and convex in shape[6]. Together, these qualities can make TFs hostile environments for the development of potent and selective drug-like small molecules. Perhaps unsurprisingly, many molecules that have been reported as direct TF inhibitors have questionable structural properties and poorly defined mechanisms of action[123,124].

However, advances in structural characterization, basic biological insights and ligand design strategies have enabled the identification of several examples of drugs and high-quality chemical probes that target TFs. Below, we review the lessons learned from examples of successful TF targeting and discuss how these insights can be applied to currently unliganded TFs.

Modulating TFs with ligand-binding domains. One of the most successful areas of drug discovery, in general, has been targeting TFs containing well-folded LBDs, namely nuclear hormone receptors. Although the specifics of targeting this class of proteins have been extensively discussed in the literature[10], here we highlight key concepts and lessons learned from decades of drug discovery with nuclear receptors.

Similar to numerous other TFs, nuclear receptors contain a DBD and a prototypical intrinsically disordered transactivation domain (known as activating function 1 (AF1))[125]. The class-defining feature of nuclear receptors is a well-folded LBD that acts as a second tunable effector domain (AF2)[125]. Binding of specific signalling molecules (for example, hormones such as oestrogen, androgen and glucocorticoids) to a LBD typically leads to activation of the TF by a variety of mechanisms including localization to the nucleus, homo-oligomerization or hetero-oligomerization and recruitment of co-activators[10,125]. Dysregulation of nuclear receptors is a feature of several cancers and other diseases, and LBDs can serve as intrinsically ligandable control points for modulating transcriptional activity. Accordingly, the drug discovery community has exploited this fact to develop many nuclear receptor drugs and chemical probes (for example, the FDA-approved androgen receptor antagonist enzalutamide) (FIG. 4).

One important realization from these efforts is that protein conformational flexibility underlies many of the regulatory mechanisms controlled by LBDs[10,126]. Ligand binding to the LBD typically activates the receptor by exposing a hydrophobic co-activator binding groove as well as, in some cases, enhancing binding to nuclear localization factors and other nuclear receptor molecules. Practically speaking, this has enabled multiple forms of modulation to be pursued (that is, agonism, antagonism and inverse agonism) for individual receptors, giving drug discovery efforts a wealth of approaches to modulate aberrant transcriptional programmes. Conformation flexibility is a common, if not central, feature of TFs[11], which suggests that tuning of TF activity by controlling conformation may be achievable for TFs as a class.

Although nuclear receptors are by far the most druggable TF family, several challenges still hamper efforts to target all family members. For example, there are many orphan receptors where the endogenous ligands are unknown or where the apparent LBD does not have a ligand binding pocket[127]. Further, in some diseases, such as castration-resistant prostate cancer, there can be expression of functional receptor splice variants that lack the LBD (ΔLBD), rendering LBD-targeting drugs ineffective[128,129]. Thus, due to the lack of well-defined and functional small-molecule binding pockets, the challenges associated with orphan receptors and ΔLBD receptor variants are more in line with the challenges of targeting other classes of TFs.

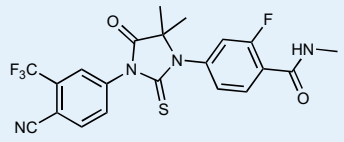
Finally, it is also becoming increasingly recognized that nuclear receptors are not the only class of TFs that contain effector domains that bind to small-molecule ligands. One noteworthy example is the TEAD family of TFs, which use a folded Yap-binding domain (YBD) to recruit co-activators in a mechanism reminiscent of the LBDs of nuclear receptors[130]. TEAD TFs are an end point of the Hippo signalling pathway, and are thus attractive therapeutic targets for cancer

Fig. 4 | **Examples of molecules that target TFs by various mechanisms.** Affinity (K_d/K_i), inhibitory activity (IC_{50}) or degradation activity (DC_{50}) are included as reported in the literature. For indirect inhibitors of transcription factor (TF) protein–protein interactions (PPIs), the molecular target is in bold. DC_{50}, half-maximal degradation concentration; HAT, histone acetyltransferase; IC_{50}, half-maximal inhibitory concentration; K_d, dissociation constant; K_i, inhibitory constant; PROTAC, proteolysis targeting chimaera.

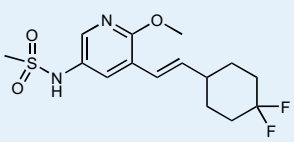
and regenerative medicine[131,132]. Notably, it has recently been determined that TEAD family members are palmitoylated at a conserved Cys residue in the YBD, which contains a deep hydrophobic pocket to bury and stabilize this typically transient PTM[133,134]. Although the specific role of the palmitoylation PTM in TEAD function remains controversial[134,135], multiple efforts have demonstrated that the palmitate pocket can be liganded by both drug-like covalent and non-covalent small molecules[136–138] (FIG. 4). For this specific TF family, significant effort is still required to develop molecules that are selective for single family members

Effector domain modulators

Direct

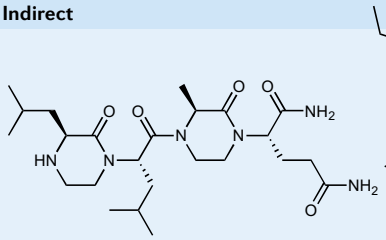


Enzalutamide, androgen receptor inhibitor
IC_{50} = 21 nM

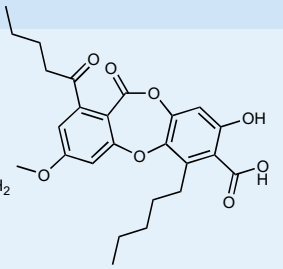


'Compound 2', TEAD inhibitor
K_d = 230 nM

Indirect



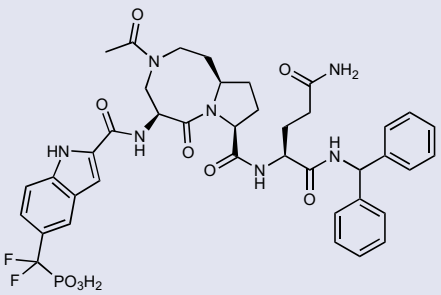
OHM1, HIFα–p300 inhibitor
K_d = 530 nM



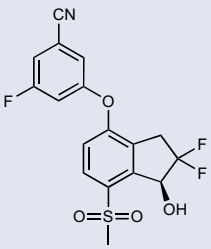
Lobaric acid, MLL–CBP inhibitor
IC_{50} = 17 μM

Regulatory domain modulators

Direct

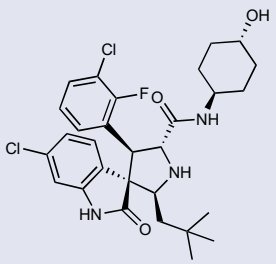


SI-109, STAT3 inhibitor
K_i = 21 nM

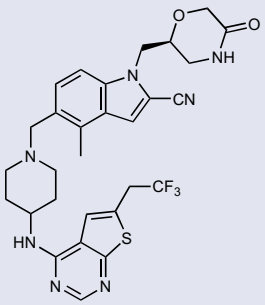


PT2385, HIF2α inhibitor
K_i = 49 nM

Indirect



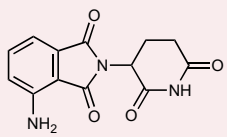
MI-77301, p53–MDM2 inhibitor
K_i = 0.88 nM



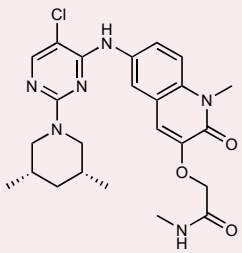
MI-1481, MLL–menin inhibitor
K_i = 3.6 nM

Degraders

Monomeric

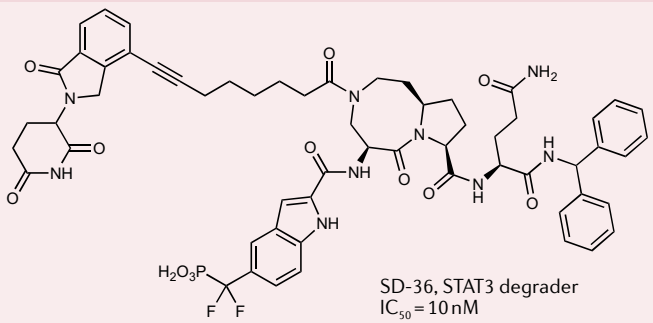


Pomalidomide, IZKF1/3 degrader
K_d = 260 nM (CRBN)



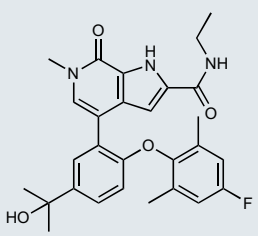
BI-3802, BCL6 degrader
DC_{50} = 20 nM

PROTAC

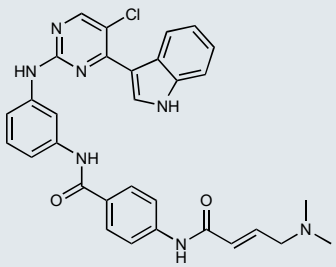


SD-36, STAT3 degrader
IC_{50} = 10 nM

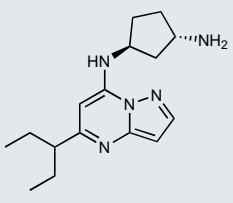
Collaborator modulators



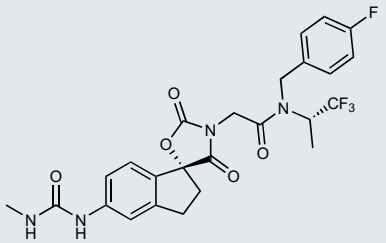
ABBV-744, BRD4 BD2 inhibitor
K_d = 2.1 nM



THZ1, CDK7/12/13 inhibitor
K_d = 3.2 nM



KB-0742, CDK9 inhibitor
IC_{50} = 6 nM



A-485, CBP/p300 HAT inhibitor
IC_{50} = 60 nM

Ubiquitin–proteasome system
A system of intracellular protein degradation that is mediated by transfer of ubiquitin to target proteins by ubiquitin E3 ligases to mark them for degradation by the proteasome.

(due to significant risks of on-target toxicity of pan-TEAD inhibitors)[132,139]. However, the results from these initial efforts indicate exciting potential for using folded effector domains with conserved small-molecule pockets as handles for expanding the druggability of TFs.

Inhibiting TF protein–protein complexes. PPIs are a key feature of TF regulation and function. Most TFs are tightly regulated by PPIs with regulatory proteins in the cytosol and/or nucleus, and similarly the basic mechanism of TF function (via effector domains) is to form PPIs with members of the transcriptional apparatus. Consequently, inhibiting specific TF PPIs is a valuable means for modulating TF transcriptional activity.

Inhibition of the p53–MDM2 interaction by small molecules serves as an excellent illustration for the potential of drugging TF PPIs[140]. In cancer, the PPI between the TF p53 — the 'guardian of the genome' — and the ubiquitin E3 ligase MDM2 often functions as a mechanism for cancer cells to evade apoptosis by downregulating p53 levels via the ubiquitin–proteasome system[141,142]. Whereas it would be exceptionally challenging to develop a small molecule that binds to p53 and stabilizes it from this mechanism of proteasomal degradation, as p53 is a highly disordered TF[143], an alternative approach is to develop antagonists of p53 binding to MDM2. The p53 binding site of MDM2 is, fortunately, highly druggable: it is a relatively small and well-defined hydrophobic pocket, which has enabled several highly potent peptide and small-molecule antagonists (for example, spiro-oxindole MI-77301) (FIG. 4) of this interaction to be developed[144,145]. These inhibitors have been shown to effectively induce apoptotic cell death in a variety of cancers by increasing p53 levels, and several are being investigated in current clinical trials[140] (TABLE 2). Given that many other important TFs are known to be regulated by specific E3 ligases, such as the hypoxia-inducible TF HIF1α by the Von Hippel–Lindau E3 ligase[146,147], there is significant potential for this approach with TFs that are aberrantly downregulated in disease.

In addition to targeting E3 ligases, developing inhibitors of other TF regulatory machinery is frequently

Table 2 | **Selected examples of TF modulators in clinical and preclinical development**

Ligand	Target	Mechanism of action	Indication	Status	Clinical trials
Direct binding to TF					
ARV-110 (Arvinas)	Androgen receptor	PROTAC	Metastatic castration-resistant prostate cancer	Ongoing phase I/II trial	NCT03888612
ARV-471 (Arvinas)	ER	PROTAC	Advanced or metastatic $ER^+/HER2^-$ breast cancer	Ongoing phase I/II trial	NCT04072952
TTI-101 (Tvardi Therapeutics)	STAT3	Inhibitor of SH2 domain	Advanced cancers including breast cancer, head and neck squamous cell carcinoma, non-small-cell lung cancer, hepatocellular cancer, colorectal cancer, gastric adenocarcinoma, melanoma	Ongoing phase I trial	NCT03195699
JPX-1188 (Janpix, Centessa Pharmaceuticals)	STAT3/5	Monomeric degrader	Acute myeloid leukaemia	Preclinical development	
PT2977 (Peloton Therapeutics, Merck)	HIF2α	Inhibitor of PASB dimerization domain	Renal cell carcinoma, advanced solid tumours	Ongoing phase I and II trials	NCT03445169, NCT02974738, NCT03634540, NCT03401788
CB-103 (Cellestia Biotech)	CSL/RBPJ	Inhibits formation of NOTCH transcriptional complex	Advanced breast cancer, advanced/metastatic solid tumours and haemotological malignancies	Ongoing phase I and II trials	NCT03422679, NCT04714619
Indirect modulation of TF					
AMG 232 (Amgen)	MDM2	Inhibitor of p53–MDM2 PPI	Acute myeloid leukaemia, advanced solid tumours, glioblastoma/gliosarcoma, metastatic melanoma, soft tissue sarcoma	Completed and ongoing phase I and II trials	NCT02016729, NCT01723020, NCT04190550, NCT03107780, NCT02110355, NCT03217266, NCT03031730
KO-539 (Kura Oncology)	Menin	Inhibitor of menin–MLL PPI	Relapsed/refractory acute myeloid leukaemia	Ongoing phase I/II trial	NCT04067336
SY-5609 (Syros)	CDK7	Inhibitor of transcriptional kinase CDK7 activity	Advanced solid tumours	Ongoing phase I trial	NCT04247126
KB-0742 (Kronos Bio)	CDK9	Inhibitor of transcriptional kinase CDK9 activity	Relapsed/refractory solid tumours or non-Hodgkin lymphoma	Phase I/II trial starting 2021	NCT04718675
ABBV-744 (AbbVie)	BRD4 (bromodomain 2 selective)	Inhibitor of transcriptional co-regulator BRD4 binding to acetylated histones	Relapsed/refractory acute myeloid leukaemia, myelofibrosis	Ongoing phase I trials	NCT03360006, NCT04454658

ER, oestrogen receptor; PPI, protein–protein interaction; PROTAC, proteolysis targeting chimaera; TF, transcription factor.

considered a promising avenue for TF modulation. For example, there is significant interest in developing inhibitors of deubiquitinating enzymes, which act in direct opposition to ubiquitin E3 ligases. Deubiquitinating enzyme inhibitors thus have the potential to destabilize overactive TFs that evade the ubiquitin–proteasome system[6,148]. The function of latent cytoplasmic TFs is often tightly regulated by PPIs with cytosolic repressors and import proteins[5], and as the methodology for drugging PPIs has advanced, these targets have become exciting avenues for developing TF modulators. Multiple modulators of the TF NF-κB, for example, have been developed by targeting PPIs involved in its activation pathways[149–151].

TFs also commonly require stable PPIs to become transcriptionally active. A prominent example of this is the STAT family of TFs, which generally require homodimerization or heterodimerization with other STAT proteins to translocate to the nucleus and activate transcription[33]. STAT dimerization is intrinsically regulated by a SH2 domain and a tyrosine residue that is phosphorylated by JAK kinases upon cytokine receptor stimulation; the SH2 domain of one STAT molecule binds to the phosphotyrosine of the other, and vice versa. Antagonists of the phosphotyrosine–SH2 interaction therefore represent a means for direct inhibition of STAT activity. Several efforts have demonstrated the ligandability of the SH2 domain, which has led to the development of potent chemical probes derived from phosphotyrosine mimetics as well as other non-peptidic scaffolds[152] (FIG. 4). Although no STAT inhibitor has yet successfully advanced through clinical trials, several inhibitors are in varying stages of clinical and preclinical development[33] (TABLE 2).

There are several other noteworthy examples where blocking stable PPIs required for TF activity has shown significant promise. The hypoxia-inducible TF HIF2α is a well-validated target for renal cell carcinoma, and potent inhibitors — including the clinical candidates PT2385 and PT2977 (FIG. 4; TABLE 2) — have been developed that block the dimerization of HIF2α with its obligatory cofactor ARNT by targeting the HIF2α PASB heterodimerization domain[153–157]. A similar approach has also been applied to the oncogenic TF MLL and various MLL fusion proteins, which are common drivers of AML. Molecules that bind the MLL cofactor menin and inhibit its association with MLL effectively abrogate oncogenic MLL transcriptional activity in cell and animal models of AML[158–162] (example structure in FIG. 4). Clinical trials are currently underway to investigate MLL–menin inhibitors as treatments for refractory and relapsed AML (for example, KO-539) (TABLE 2). Finally, dysregulated NOTCH signalling is implicated in a wide variety of cancers[163], and it was recently shown that NOTCH transcriptional activity can be effectively abrogated by a small molecule (now clinical candidate CB-103) (TABLE 2) that binds to the TF CSL/RBPJ and inhibits association with the NOTCH intracellular domain[8]. Importantly, preclinical data highlight a therapeutic advantage of directly targeting CSL/RBPJ, as the gastrointestinal toxicity commonly associated with upstream NOTCH inhibitors is not observed with CB-103 (REF.[8]).

Critical TF PPIs can also be modulated by stabilizing or destabilizing repressed forms of the TF in the nucleus. For example, the fusion protein CBFβ–SMMHC drives some forms of AML by homodimerizing and sequestering the RUNX1 TF from target DNA sites[164], and effective dimeric inhibitors have been developed to selectively inhibit CBFβ–SMMHC dimers and restore active RUNX1 (REF.[165]). Conversely, it has recently been shown that inhibition of MYC activity can be achieved by stabilizing transcriptionally incompetent homodimers of its requisite binding partner MAX with small molecules, leaving MYC unable to form a functional DBD and causing it to be rapidly degraded[166].

In addition to stable PPIs with cofactors, TF transcriptional activity is largely dictated by recruiting co-activators to specific genomic loci, making TF–co-activator PPIs intriguing targets for controlling TF activity. Whereas many co-activators function as general transcriptional hubs, which could raise doubts about the level of selectivity achievable with this strategy, many co-activators such as CBP/p300 or Mediator contain multiple distinct and usually well-folded activator binding domains (ABDs) that recognize specific subsets of TFs via their transactivation domains[15,24]. Thus, targeting individual ABDs may be an effective avenue for selective inhibition of TF activity. Major challenges with this approach, however, are that these PPIs tend to be considerably more dynamic and transient than PPIs between TFs and cofactors or regulatory proteins, and the functional binding surfaces of the ABDs are relatively large and shallow. However, advances in peptidomimetic strategies[167,168] and increasing data indicating the highly allosteric nature of ABDs[169,170] have enabled some progress against these targets. For example, moderately potent oligooxopiperazine α-helix mimetics have been developed for the TAZ1 domain of the co-activator CBP/p300 (REF.[167]) (FIG. 4), and natural products such as lobaric acid (FIG. 4) have been discovered to allosterically inhibit the CBP/p300 KIX domain[171,172]. Although outside the scope of this review, peptide-based strategies have also shown promise for targeting TF–co-activator PPIs[173,174]. The future will hopefully see the continued development of more potent and selective chemical probes of TF–co-activator PPIs.

Modulating stability with molecular glues and monomeric degraders. One exciting avenue for targeting TFs that is currently making clinical impact is the development of molecular glues and/or monomeric degraders that directly control TF stability. Molecular glues function by inducing non-native PPIs between proteins and have been described in the literature for decades, but until recently were thought of as rare quirks of natural products[175]. However, it has been increasingly observed that molecular glues are a relatively frequent mechanism of action for natural and synthetic bioactive molecules[175]. A watershed moment in the field was the discovery that the clinically approved thalidomide-based anticancer immunomodulatory imide drugs (IMiDs) (FIG. 4) function by inducing non-native PPIs between Ikaros zinc-finger (IKZF) TFs and the E3 ligase CRBN, leading to degradation of IKZFs by the ubiquitin–proteasome

Molecular glues
Small molecules that directly mediate a non-native protein–protein interaction.

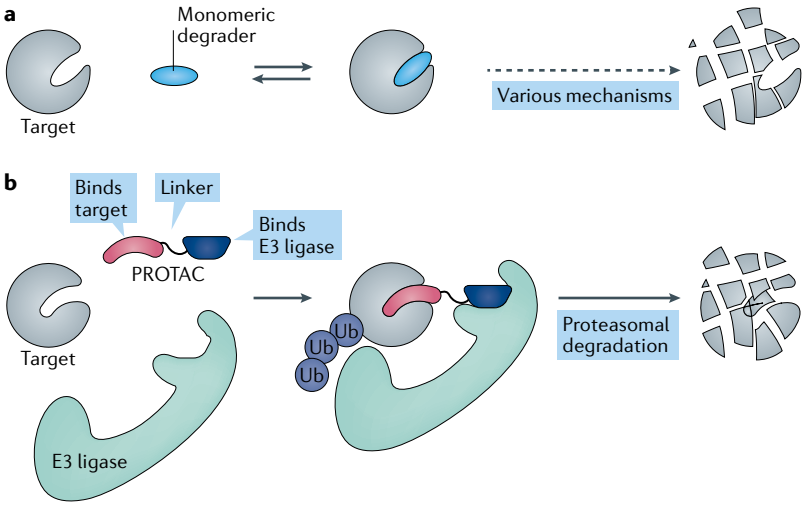


Fig. 5 | **Overview of monomeric and PROTAC-based targeted protein degradation strategies. a** | Monomeric degraders function by binding the target of interest and inducing its degradation through a variety of mechanisms (for example, inducing neo-protein–protein interactions with ubiquitin E3 ligases in the case of immunomodulatory imide drugs (IMiDs))[178]. **b** | Proteolysis targeting chimaera (PROTAC) degraders co-opt the ubiquitin–proteasome system by linking a binder of the target of interest (red) to a ligand for a ubiquitin E3 ligase (purple), which induces catalytic ubiquitylation and degradation of the target protein.

system[176–180]. Critically, IMiDs overcome the challenge of directly binding to a disordered TF by, instead, binding first to CRBN and then stabilizing an interface that recognizes a conserved IKZF loop[180,181]. Other TF molecular glues have been reported and include the manumycin family of polyketides, which form a covalently linked PPI between p53 and the E3 ligase UBR7 (REF.[182]). Interestingly, this neo-interaction between an E3 ligase and a potential substrate results in stabilization and activation of p53, rather than degradation.

The discovery of TF-targeting molecular glues has also overlapped with the enthusiasm about developing monomeric degraders — molecules that directly bind a protein and induce its degradation — as a novel strategy for directly modulating TF activity[183]. The possibility of developing effective monomeric degraders became apparent after the realization that the FDA-approved oestrogen receptor (ER) antagonist fulvestrant works by binding to the receptor LBD and inducing proteolytic degradation[184,185]. This mechanism of action lends considerable therapeutic benefits over traditional ER antagonists because of the increased and prolonged efficacy associated with elimination of ER from the cell. In addition, the catalytic elimination of ER by monomeric degraders can enable the ability to overcome resistance mutations that would decrease the occupancy of traditional LBD modulators. Because monomeric degraders rely on direct binding to TFs, development has largely focused on selective degraders of highly ligandable nuclear receptors (that is, androgen and oestrogen receptors)[183], although there are select examples of monomeric degraders of other TFs[186,187].

Interestingly, it is possible that many monomeric degraders work by molecular glue-type mechanisms. During the development of inhibitors that block homodimerization of the BTB effector domain of the TF BCL-6, it was discovered that the most potent inhibitors actually functioned as monomeric degraders that drastically decreased the protein levels of BCL-6 (REFS[186,187]). Detailed mechanistic work showed that one of these monomeric degraders (BI-3802) (FIG. 4) functioned by a molecular glue-type mechanism, where it induces self-association of BCL-6 into polymeric fibrils that are subsequently degraded by the ubiquitin–proteasome machinery[188]. Thus, there is probably significantly more to discover about the possible ways that molecular glues and monomeric degraders function, which could likely enable these approaches to take on more TFs.

An issue that hampers the development of molecular glues and monomeric degraders is that it is still highly challenging to rationally design or discover molecules that work by these mechanisms. Indeed, most examples of molecular glues and monomeric degraders were originally discovered by phenotypic assays or developed as traditional antagonists[183]. Significant effort is underway to mechanistically dissect more of these molecules and to develop targeted approaches to discover additional glues or degraders, which together will enable more rational approaches to be developed[188–191].

Degrading TFs with proteolysis targeting chimaeras. PROTACs offer a more rational way to design TF degraders[192–194]. PROTACs are bivalent molecules comprising a ligand that binds to a target protein linked to a ligand that recruits a ubiquitin E3 ligase (FIG. 5). Similar to the IMiD drugs, PROTACs function by inducing a neo-interaction between the target protein and an E3 ligase that leads to degradation of the target protein by the ubiquitin–proteasome system. However, in contrast to molecular glue and monomeric degraders, PROTACs are modular and, in theory, any ligand that binds to a target protein has the potential to be turned into a degrader.

PROTACs have several advantages over standard occupancy-based antagonists that make them an especially enticing modality for targeting TFs. Because TFs frequently contain several distinct protein domains of varying functional activity, antagonists of single domains are often not sufficient to effectively inhibit TF activity[195]. By degrading a target protein, a PROTAC inhibits all of its functions rather than just the activity of a single domain[196–199]. PROTACs also gain several benefits from their catalytic mechanism of action, including an increased durability of response from removal of the target protein and lowered susceptibility to resistance mutations that decrease affinity of the target ligand[200–204]. Further, families of TFs frequently contain a substantial degree of sequence conservation that makes the optimization of target selectivity for traditional inhibitors a challenging task. Because PROTAC selectivity is not governed by molecular recognition alone but by a combination of ternary complex formation, protein turnover rates and availability of target Lys residues for ubiquitylation, even highly promiscuous ligands have been shown to make surprisingly selective degraders[205–209]. Together, several of the traditional challenges associated with developing TF modulators have the potential to be overcome by PROTAC degraders.

Histone deacetylases
(HDACs). Enzymes that remove acetyl groups from acetylated Lys residues in histones. Generally associated with closed chromatin conformation and transcriptional repression.

Histone acetyltransferases
(HATs). Enzymes that transfer acetyl groups to the ε-amino group of Lys residues in histones. Generally associated with open chromatin conformation and increased transcription.

Chromatin remodellers
Protein complexes with a common ATPase domain that use ATP hydrolysis to move, reposition or eject nucleosomes.

Intrinsically disordered proteins
(IDPs; also known as intrinsically disordered regions (IDRs)). Proteins or regions of proteins that do not adopt a well-defined structure. Often characterized as 'ensembles' of many unrelated protein conformations, although many IDPs/IDRs can transiently form more defined structures.

NMR spectroscopy
A structural technique that utilizes the quantum-mechanical properties of nuclear spins in a magnetic field. Used in structural biology to determine protein structure, as well as to characterize conformational dynamics across a wide range of timescales (general range of picoseconds to hours/days).

Molecular dynamics
A computational technique that is used to characterize the structure and conformational dynamics of proteins by simulating the interactions between all the atoms of a protein and its surrounding solvent over time.

Occupancy-based modulators
Modulators of protein function in drug discovery where the overall change in protein activity from drug treatment is determined by the concentration of the drug in the cell and its affinity for its target. For complete inhibition of activity by an inhibitor, the drug must reach concentrations several times above its dissociation constant (K_d).

Widespread recognition of the promise of targeting TFs with PROTACs is illustrated by the fact that the first PROTAC degraders entering into clinical trials target the androgen and oestrogen nuclear receptors[210] (ARV-110 and ARV-471) (TABLE 2). Despite concerns about the pharmacokinetic/pharmacodynamic properties of large PROTAC molecules[211], preliminary data have indicated that these molecules can still be effective even in otherwise refractory tumours. Several other PROTACs for TFs and TF co-regulators have also been developed that show additional benefits over occupancy probes, including degraders of STAT3 (REF.[195]), MDM2 (REF.[212]) and BRD4 (REFS[213,214]) (for example, the highly effective STAT3 PROTAC SD-36) (FIG. 4).

Blocking aberrant transcriptional activity via TF collaborators. One rather surprising insight from recent studies of TF action is that chemical perturbation of individual general transcriptional cofactors such as transcriptional kinases, epigenetic proteins and co-activators can be highly specific to cell-identity transcriptional programmes. This has been linked to the fact that cell-identity genes are frequently controlled by super-enhancers, which are typically more sensitive to inhibition of general cofactors over typical enhancers due to their extraordinarily cooperative nature[51,53,56,96]. For example, it has been shown that inhibition of chromatin readers (BRD4)[56,95,96,215,216], transcriptional kinases (CDK7, CDK9, CDK12)[58,59,217], histone modifying enzymes (histone deacetylases (HDACs), histone acetyltransferases (HATs) and so on)[60,61,218] and chromatin remodellers (BAF, CHD4)[62,219] can be quite specific to super-enhancer gene expression. A high degree of selectivity for specific super-enhancer-driven transcriptional programmes has even been observed for the CDK9 inhibitor KB-0742 and the BRD4 bromodomain 2 (BD2) inhibitor ABBV-744 (REFS[59,215]) (FIG. 4), which is likely caused by variation in the cofactor composition of individual super-enhancers based on differing recruitment preferences of individual TFs. Therapeutic windows for general transcriptional cofactor inhibitors may therefore be unexpectedly large. Together, this indicates that targeting TF collaborators is a promising avenue to selectively inhibit dysregulated transcription in diseases driven by super-enhancers, such as cancer.

Ongoing challenges, emerging solutions

Although there have been many successes in developing TF modulators over the past two decades, there are still several obstacles in the way of targeting more members of this protein class. Below, we outline current hurdles for targeting undrugged TFs and new strategies and technologies that have the potential to overcome these barriers.

Directly targeting TFs without traditionally ligandable domains. The vast majority of well-validated and potent small-molecule modulators that directly bind TFs target those that contain either a small-molecule binding domain (nuclear receptors, TEADs)[10,130] or a structured dimerization domain (STATs, HIF2α, BCL-6)[79,152,153,186]. In the case of the IMiD molecular glues, the primary binding site of the molecule resides in a well-defined pocket in the non-TF partner[176,179]. Successfully targeting TFs has therefore relied heavily on the presence of well-defined protein folds with ligandable pockets. However, a shared feature of TFs is that they contain a significant degree of structural disorder[11], and outside the cellular context many TFs are predicted to be almost entirely intrinsically disordered. This is a significant impediment for modern targeted drug discovery strategies that heavily rely upon structural information to guide ligand optimization, such as fragment-based drug design[220,221].

Recent advances in our basic biophysical understanding of intrinsically disordered proteins (IDPs) along with the development of experimental techniques to characterize IDP structure hold significant promise for overcoming the many challenges imposed by TF disorder. For example, despite their historical portrayal as floppy noodles completely devoid of three-dimensional structure[28], IDPs display a significant degree of structural variation. Depending on the amino acid sequence, IDP structures vary from extended dynamic chains, to condensed but disordered globules, all the way to structural ensembles containing transient but well-defined folds[222–227]. Many disordered TFs, especially in the latter category, may therefore contain transiently formed pockets that could be exploited to develop ligands.

Realization of this goal of developing ligands for disordered TFs will require the adoption of experimental techniques that are highly suited to IDP characterization. Standard structural techniques such as X-ray crystallography and cryo-electron microscopy unfortunately fare exceptionally poorly at resolving disordered regions. On the other hand, the combined use of NMR spectroscopy and molecular dynamics approaches has been shown to be highly suited for characterizing IDPs at the atomic level, and thus could serve as excellent tools to identify and characterize transient pockets and their interactions with ligands[228–231]. Additional approaches with these techniques for characterizing IDP–ligand interactions will be very important, as unoptimized screening hits for IDPs will likely bind in a highly dynamic manner that makes successful ligand optimization challenging[123,232,233].

Targeted protein degradation. The majority of reported TF PROTACs are derived from ligands developed to target either nuclear receptor LBDs or TF protein–protein interfaces[195,234–237]. This reliance on previously developed ligands results in a strong bias of TF PROTAC development towards already druggable TFs. There is therefore significant untapped potential of this technology for targeting currently intractable TFs, and several aspects of PROTAC design and mechanism may actually make them especially suited for challenging TFs over traditional occupancy-based modulators.

A sometimes underappreciated fact about PROTACs is that they obviate the requirement for occupancy-based ligands to not only bind to a protein but also modulate its function. That is, a small-molecule binder can target any region of a protein — even functionally inactive domains — and still be able to serve as an effective PROTAC because the E3 recruiting module produces the functional effect[197,199,238]. Furthermore, large-scale

ARV7
A splice variant of the androgen receptor that lacks the ligand-binding domain.

proteomics studies with kinase degraders have shown that even relatively weak binders of the target protein can still make highly potent PROTACs[200,205,209]. Together, these characteristics have the potential to overcome the major challenges and trade-offs associated with designing potent and functional modulators of TFs. Ligand development efforts for PROTACs can therefore focus on optimizing lead binders without the additional constraints of activity and/or affinity thresholds necessary to be viable for use as therapeutics or chemical probes. A recent example that highlights the potential of this approach is the development of effective PROTACs directly targeting the 'undruggable' androgen receptor ΔLBD splice variant ARV7 from ligands that moderately inhibit the androgen receptor DBD[239,240]. Together with potential advances in developing ligands of dynamic and disordered regions, the PROTAC approach could open up a significant portion of TF target space.

However, several challenges still stand in the way of expanding the repertoire of TF PROTACs[241]. Not all E3 ligases can effectively degrade a given protein, and there are currently only a handful of E3 ligase recruiting ligands to choose from[213,214,242,243]. Current efforts are underway to add additional E3 ligases to the PROTAC toolset. An especially exciting preliminary development is the discovery of covalent E3 binders that irreversibly convert an E3 ligase into a destruction complex targeted towards a specific protein[244–248]. Additionally, it is exceptionally challenging to optimize non-functional small-molecule binders of TFs for use as PROTAC handles, as there are very few quantitative direct binding assays that can be used in the likely essential context of the cell[249]. Finally, there is considerable need to develop workflows to rapidly assess which ligands can be turned into effective PROTACs to minimize the time spent optimizing scaffolds that are not well suited for development into PROTACs[250].

Binding-focused screening. Binding-focused screening strategies[251] are increasingly being recognized as highly promising methods for the discovery of TF modulators. These approaches are especially relevant to the development of PROTAC strategies for TFs, which can use even non-functional binders as the foundation for effective degraders. Two technologies have demonstrated significant potential for targeting TFs: small-molecule microarray (SMM) and covalent screening approaches[252–254].

The SMM screening approach utilizes libraries of small molecules covalently anchored to glass slides as an extremely rapid, versatile and functionally agnostic way to screen for small molecule–protein interactions[252,253]. Critically, interactions between putative small-molecule binders and a protein target can be detected by fluorescent antibodies specific to the protein or an added epitope tag. This enables screens to be run against not only purified proteins but also specific proteins in highly complex environments such as cellular lysates[252]. The latter can even be exploited to screen endogenous proteins from disease-relevant cell lines. Ligands for challenging TFs such as ETV1, SHP and MAX have been discovered using SMM screening, supporting the potential of this technique for directly targeting TFs[166,255,256]. Furthermore, SMM screens using cellular lysates have the potential to identify ligands of critical TF binding partners and collaborators. This was recently illustrated by the discovery of a selective CDK9 inhibitor from a lysate screen against the 'undruggable' androgen receptor splice variant ARV7 (REF.[59]). SMM screening is thus an exceptionally useful approach for drugging TFs and their collaborators, that does not require significant protein engineering or prior knowledge to implement effectively. However, one potential drawback to this approach is that it often requires significant effort to dissect the mechanism of action of candidate molecules identified by SMM screens.

Covalent screening approaches use libraries of molecules with reactive warheads — most often Cys-reactive moieties such as acrylamides or chloroacetamides — to screen for covalent binding of molecules to a target protein[254,257,258]. Mass spectrometry is typically used to identify covalent binders, enabling significant versatility for this approach. For example, although it is most common to screen covalent libraries against purified proteins, it is also possible to screen for covalent binding in live cells so long as the target of interest is detectable by the mass spectrometry technique used[254,257]. Excitement for this area of drug discovery, in general, has been increasing since the discovery of covalent ligands and clinical candidates for the 'undruggable' oncogenic KRAS-G12C mutant[259–261], and many consider it an approach well suited for challenging targets such as TFs. Supporting this idea, unbiased screening of Cys-reactive small-molecule libraries in living cells has identified previously unknown ligandable sites in many undrugged TFs[254]. The recent identification of a covalent MYC inhibitor that selectively binds to a MYC IDR suggests that covalent approaches may even have the potential to overcome the traditional challenges associated with targeting heavily disordered proteins[262]. There is thus significant potential for covalent approaches to open further TF target space. However, it bears noting that target identification is an absolute critical step of covalent inhibitor development because of the possibility of off-target reactivity driven by the warhead, and it is still challenging to conduct targeted screens in cells with low-abundance or unstable proteins.

Finally, one of the most popular binding-focused screening approaches in recent years has been DNA-encoded libraries (DELs), which are massive libraries of small molecules that are tagged with individual DNA 'barcodes'[263–265]. Because DELs are generated combinatorially and individual molecules are directly attached to a DNA record of all chemical transformations performed on the scaffold, it is possible to screen mixtures of billions of compounds at once by using PCR amplification to detect binders. This approach is therefore especially appealing for TFs, which are typically characterized by very low hit rates in traditional screens. New approaches to synthesizing DELs are even being developed to significantly increase the chemical diversity of molecules in individual screening sets, which is historically a weakness of DELs[266,267]. However, this approach has not yet realized significant success with TFs. A key

challenge is that DEL screening with TFs is currently relegated to purified protein screens because the requisite DNA tag interferes with cellular penetration. TFs that adopt structure only in the presence of binding partners will thus be more difficult to screen effectively. Further, screening full-length TFs by this approach also poses a challenge because non-specific binding of DBDs to the DNA barcodes can interfere with the results. Nonetheless, the DEL approach has significant potential for generating chemical matter against difficult targets, and its use for TFs should be encouraged.

Target engagement and selectivity. Key to the development of targeted therapeutics and chemical probes, in general, is being able to tell that a molecule produces its phenotypic effects via engagement with the intended target and not by any number of off-target mechanisms[268]. Many 'undruggable' TFs are especially challenging in this regard due to difficulties with obtaining high-quality structural, biophysical binding and cellular engagement data[123,255,262]. Unsurprisingly, many putative TF inhibitors contain obvious pan-assay interference scaffolds such as rhodanines, quinones and/or other liabilities that could have been readily deprioritized or ruled out with the use of pan-assay interference scaffold filters or reliable biophysical and cellular validation assays[269–271]. Furthermore, because inhibition of general transcriptional cofactors can, in some cases, phenocopy TF inhibition profiles[59,62,215], it is essential to obtain unequivocal evidence that a molecule functions through direct engagement with a TF.

Several technologies have evolved in recent years to take on the challenges of determining target engagement and selectivity for small-molecule ligands. Direct binding approaches have been an area of significant impact with the development of cellular thermal shift assays (CETSAs) and photoaffinity labelling approaches[249,272–274]. Proteome-wide CETSAs essentially measure shifts in the melting temperature of proteins in the cell upon treatment with a ligand, which indicates likely molecular targets. Photoaffinity labelling approaches make use of photolabels attached to a ligand to form a covalent bond between the ligand and target protein(s) when treated with UV light. Both of these techniques can be used in living cells, giving an unprecedented look at the global target profile of a ligand. However, both techniques have drawbacks to consider. Whereas the CETSA is one of the most straightforward and practical techniques to perform, it is prone to false positives because perturbation of PPIs can also cause thermal shifts in the binding partners of the true target[262,275]. Given that TFs spend much of their time in complex with other proteins, this must be considered when evaluating any CETSA-based TF engagement data. On the other hand, photoaffinity labelling can give unequivocal evidence of direct binding because the photolabel (typically a diazirine) forms a strong covalent bond to the molecular target(s), but the technique requires modification of the ligand to add the photoaffinity label. In addition, photoaffinity labelling approaches have the potential to miss 'true' targets due to uneven amino acid reactivity preferences of the photolabel[276].

For PROTACs and covalent ligands, cellular target identification is generally rather straightforward because of the ability to use proteomics approaches. PROTACs function by degrading their targets, and therefore proteomics can be directly used to identify targets that are downregulated by PROTAC treatment[205,209]. However, a significant drawback is that if the degrader is slow-acting, then it may be challenging to determine whether it is degraded directly by the PROTAC or is indirectly downregulated by other means[209]. For Cys covalent ligands in particular, specific proteomics technologies have been developed to enable proteome-wide screens of free Cys residues. The most widely used technique is isotopic tandem orthogonal proteolysis-enabled activity-based protein profiling (isoTOP ABPP), which assays all reduced Cys residues that can react with a non-selective iodoacetamide ABPP probe[254,262,277,278]. Targets of Cys-reactive ligands can thereby be identified by a reduction in labelling by the ABPP probe upon pretreatment by the ligand. One remaining challenge with this approach is that the ABPP probe cannot currently detect all reduced Cys residues in the proteome and the technique can be especially difficult to implement with unstable or low-abundance proteins[262].

Functional target identification approaches have also become an area of significant innovation that bear consideration for use in any TF ligand discovery campaign. Classically, the gold standard of target identification is to test ligands against cell lines bearing point mutations of the putative target that inhibit molecule binding without affecting function, because this directly relates binding of a ligand to its functional effects[279]. However, this approach can be quite challenging for TFs outside nuclear receptors because the ligand binding sites are often challenging to identify and characterize, making generation of effective inhibitory mutations difficult. Significant potential for rapid target identification has recently been demonstrated by unbiased CRISPR dependency screening, to identify targets that confer effectiveness to a ligand[280,281]. However, for TFs, results from knockdown-based screens must be taken with caution because cells that are addicted to the target TF's activity may not tolerate its knockdown.

Together, the target engagement and identification approaches described in this section represent imperfect but invaluable tools for TF drug discovery campaigns. Given the historical challenges associated with developing selective TF modulators, use of one or ideally several of these approaches should be considered essential for the development of any new TF-targeting ligand.

Targeting phase-separated condensates. The emergence of data linking transcriptional activation to the formation of phase-separated transcriptional condensates[13,37] naturally invokes questions about how the physicochemical properties of condensates could influence the action of TF-targeting ligands. Indeed, in vitro studies have indicated that drugs and drug candidates that target TF and/or TF co-regulators often have significant preferences for entering transcriptional condensates over other types of condensates, even in the absence of the ligand binding site in the target protein[282]. It is therefore

pertinent to evaluate the physicochemical properties associated with partitioning into condensates, which could have a significant effect on a ligand's ability to reach its target. Moreover, it will be extremely important to gain an understanding of the circumstances where entering a condensate is required for drug action. For certain approaches, it may even be advantageous to engage a TF outside transcriptional condensates; for example, a PROTAC that preferentially partitions into transcriptional condensates may have difficulties reaching an E3 ligase outside the condensate. At present, there are many more questions than answers regarding the relationship between condensates and drug action, but future insights may have significant impact on the ways we approach TF-targeting and ligand design.

Outlook

The field of targeting TFs has come a long way since James Darnell penned his eponymous call to arms almost two decades ago[5]. Indeed, some of the TFs he nominated as appealing targets (for example, STAT TFs) have seen significant drug discovery efforts, with promising molecules in preclinical development or entering clinical trials (TABLE 2). Yet there are still many more TFs that have not been successfully targeted. In the near term, innovations in identifying and targeting TF collaborators and the continued development of TF PPI inhibitors (especially for TF regulatory domains) appear to have the most significant translational potential to deliver additional TF modulators and drugs.

However, even with these more tried and true approaches, there are still major challenges associated with targeting TFs as a class that must be addressed over the longer term. Even for well-studied TFs, there are still many mysteries about the molecular determinants of function that produce roadblocks to rationally devise ways to effectively drug them. For understudied TFs, this challenge is made orders of magnitude more difficult. Therefore, it is essential to adopt or develop approaches that suit the specific challenges of this target class to enable continued success in this field.

One excellent example of a set of related approaches that appear to be highly suited to TF modulation are proximity-inducing bifunctional molecules such as PROTACs. The induced proximity strategy, by essentially splitting ligand binding and function, can negate the need to identify and target functionally relevant surfaces of TFs. Although this approach has been relegated to developing PROTACs for already druggable TFs, it can and should be used for currently intractable TFs. Looking towards the future, the addition of novel induced proximity approaches to the chemical biology toolbox, for example phosphorylation-inducing chimaeric small molecules[283], may even enable these approaches to have more fine-tuned control over TF action.

Additionally, the pervasive level of intrinsic disorder in TFs is still a significant hurdle to developing potent ligands. Unbiased binding-based approaches can help to uncover the ligandable sites of TFs, especially when coupled with extensive biophysical dissection to determine the binding modes of promising ligands. Indeed, there are several promising hints that some IDRs may indeed be ligandable. For example, drug-like direct binders of the disordered TFs ETV1 (REF.[255]), EWS–FLI1 (REF.[284]) and MYC[262], along with binders of disordered AF1 regions of nuclear receptors[285,286], have been reported in the literature. Unfortunately, in some of these reported cases, the functional link between the ligand-produced phenotype and IDR binding has not been fully elucidated, underscoring the need for rigorous target engagement and identification studies on all putative TF binders.

Although once thought of as almost entirely undruggable, it has become clear that, with the right set of tools and sustained effort, many TFs can be successfully targeted. With several promising strategies emerging, the future holds exhilarating potential for drug discoverers to access new areas of the TF target class. Moreover, given the foundational role TFs play in numerous diseases, the benefits to patients from drugging TFs cannot be overstated.

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