Transcription factors: a new frontier for drug discovery

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Altered gene expression is fundamental to the etiology of many human diseases, including cancer, cardiovascular disease, neurologic and immune disorders. Nuclear transcription factors are acknowledged to play a key role in these diseases through their role as regulators of tissue- and stimulus-specific gene expression. Rapid advances in our understanding of the fundamental molecular biology of transcription factors, and the signal transduction mechanisms that regulate their activity, have pioneered a new frontier for the rational development of drugs that selectively regulate gene expression. These advances promise new opportunities for the treatment of significant and largely unmet human diseases.

The discovery of therapeutic agents has traditionally relied upon random screening of large chemical libraries for compounds that interfere with biological events associated with disease. The productivity of this traditional drug discovery paradigm began to falter during the past two decades, due primarily to the application of more stringent government regulations requiring increasingly prolonged development processes for therapeutics for which the mechanism of action was poorly understood. A new, more definitive drug discovery process has evolved out of the revolution in our understanding of the molecular basis of disease, brought about in large part through the advent of molecular biology. With this new era came the riches of recombinant DNA technology, molecular and cellular biology, and the discipline of molecular medicine, where one can not only define an individual as the sum total of a series of genes arranged on a finite number of chromosome scaffolds, but where one can also systematically define those molecular defects that lead to many of the major diseases afflicting humans.

Our enhanced understanding of the molecular basis of a diverse range of human diseases leads to the appreciation that alterations in the expression of specific genes are fundamental to the disease etiology. Cancer, cardiovascular disease and neurologic, inflammatory and immune disorders all arise to a large extent from overproduction or underproduction of one or more proteins. The role of transcription in the aberrant production of disease-related proteins has become apparent. Increasing knowledge of the specialized proteins that regulate the transcription of disease-related genes has paved the way for a new drug discovery process – that of targeting individual genes and their regulators as a means of regulating disease progression.

This review attempts to summarize our understanding of the key role that nuclear transcription factors play in human disease and to demonstrate how advances in the basic molecular biology of these molecules have expanded the potential for rational drug design.

The role of transcription factors in gene expression

Architecture of a transcription initiation complex

The information required for the synthesis of a specific protein is encoded in the sequence of nucleotides in DNA. This information must be transcribed into RNA, the messenger molecule, which is then translated in the cytoplasm by the

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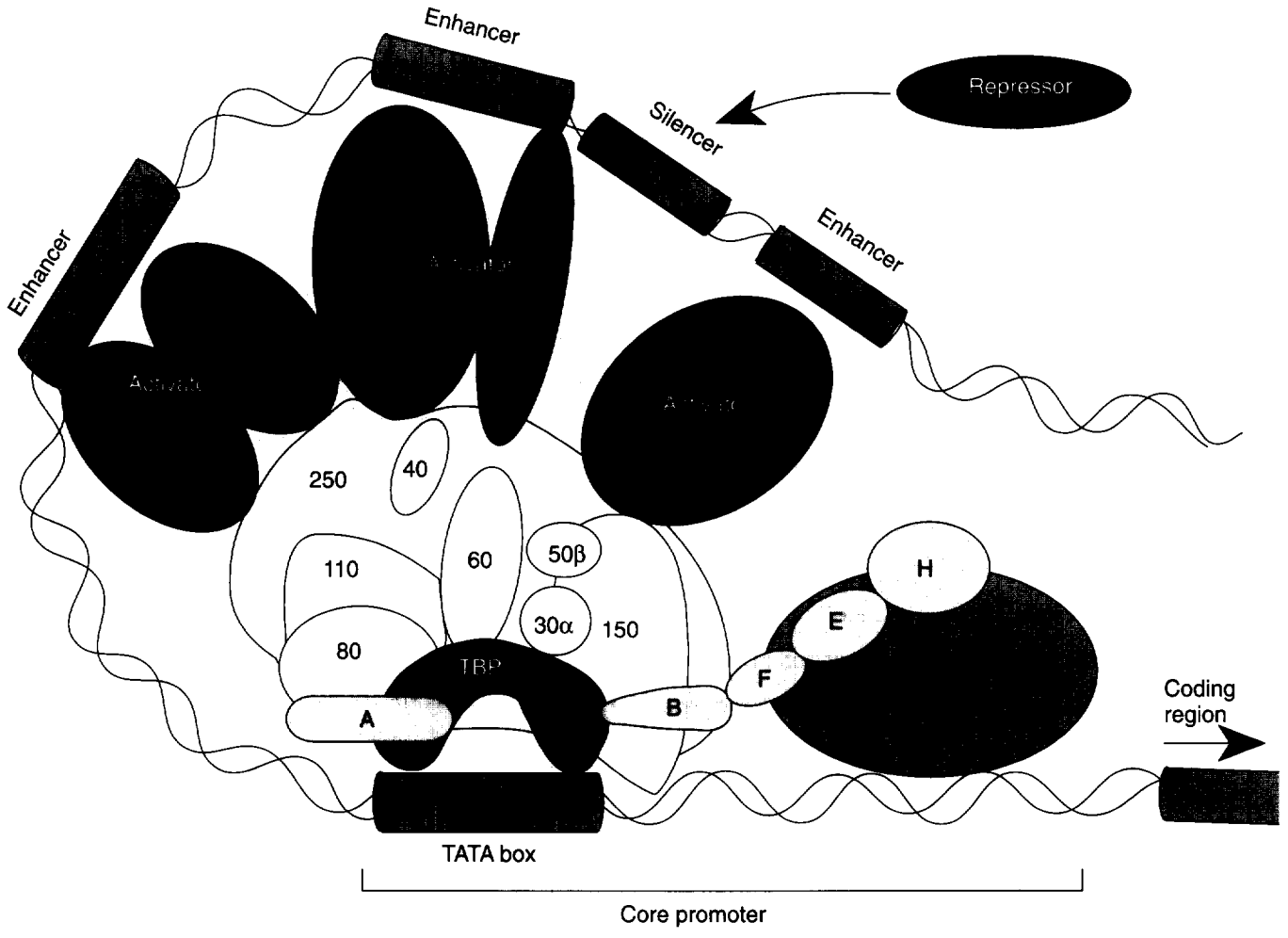

Figure 1. *The molecular architecture of the transcription complex regulating RNA Pol II activity in eukaryotes. Basal factors (A–H) and the TATA-box-binding protein (TBP) bind to the core promoter region of a gene and can modulate the activity of Pol II. A number of coactivator proteins (identified by their molecular weights) are also linked in a tight complex with TBP. Activators and repressors can communicate with the basal factors through interaction with different coactivators. Activators and repressors are commonly referred to as transcription factors.*

protein synthesis machinery present in all cells. Specialized enzymes called RNA polymerases catalyze the process of transcription of DNA into RNA. Of the three different RNA polymerases found in mammalian cells, RNA polymerase II (Pol II) serves to transcribe the majority of genes[1]. This protein binds in a region of DNA upstream of the translation initiation codon and is referred to as the promoter. There are many accessory proteins that can interact with Pol II to influence its transcriptional activity, with upwards of 50 individual proteins being identified as part of the complex that drives the transcription of most, if not all, human genes[2] (Figure 1). These proteins can be defined as belonging to one of four different groups, broadly defined by their different roles in the construction of a transcriptional complex. Basal factors, generally named by single letters, are essential for transcription but cannot by themselves increase or decrease its rate. That task falls to regulatory molecules known as transcriptional activators and repressors. Activators, and possibly repressors, communicate with the basal factors through coactivators; these coactivators are proteins that are linked in a tight complex to the TATA-box-binding protein (TBP), the first of the basal factors to bind to the core promoter region of genes. Coactivators are named according to their molecular weight (kDa). Transcriptional activators, which interact with Pol II through this non-covalent mechanism, are commonly referred to as nuclear transcription factors (TFs).

TF– DNA binding

TFs bind to double-stranded DNA, mostly in regions upstream of the core promoter, that are commonly referred to as enhancer elements. They bind to short-sequence motifs of variable length, usually 5–20 bp, which are generally specific to a single TF or to the members of a single TF family[3]. TFs can bind singly or as homodimers to a specific motif; however, in many cases they bind as heterodimers with other members of their family or with members of other families of TFs. TFs are composed of multiple domains, including surfaces for interaction with DNA, sequences involved in transcription activation and domains for multimerization and response to physiological and extracellular signals. The known TFs fall into six major families, classified according to similarities in their DNA-binding and/or transactivating domains[4] (Table 1). Each of these families binds DNA by distinct mechanisms, effectively increasing the potential for specificity in the recognition of enhancer or silencer sequences in the promoters of different genes.

It should be noted that more than 300 TFs present in the vertebrate genome have been identifed to date[5]. With the rapid progress in gene identification as part of the human genome sequencing efforts, it is likely that the number and size of TF families will expand significantly, and with it the potential for identifying new targets for drug discovery.

Regulation of transcription factor activity

It is not possible to appreciate the role of TFs in disease progression without understanding the mechanisms that regulate their activity. The ability of specific TFs to bind DNA and modulate the transcriptional activity of a given gene is highly regulated in a normal cell. Just as there are many different TF families, so too are there many different mechanisms that regulate TF activity. These mechanisms are highly diverse, yet display an extraordinary degree of specificity for a given TF (Figure 2).

Cytoplasmic inhibitors

Many TFs reside within the cytoplasm of normal cells, retained there by the binding of an inhibitory subunit. This physical compartmentalization restricts access to the nucleus, thus preventing DNA binding and transcriptional activation. An appropriate stimulus, usually the binding of a soluble factor to a specific cell-surface receptor, will activate a signal transduction pathway which removes the inhibitor from the TF, thus facilitating nuclear translocation. Members of the NF-κB–Rel family represent examples of TFs regulated in this fashion[6]. In resting cells, NF-κB is bound by an inhibitory subunit known as IκB-α. Binding of IκB-α effectively masks the nuclear localization sequences present on the p50 and p65 subunits of NF-κB, thus preventing nuclear translocation. Upon cellular stimulation, a signal transduction pathway is activated, leading to phosphorylation of key serine residues in the N-terminus of the IκB-α polypeptide. IκB-α phosphorylation serves as a molecular tag to initiate the selective proteolytic degradation of IκB-α by the ubiquitin–26S proteasome system[7]. NF-κB is then able to translocate to the nucleus where it plays a key role in the transcriptional activation of a wide range of inflammatory genes[8].

One of the genes activated by NF-κB is the IκB-α gene; increased production of IκB-α in the cytoplasm serves as a negative feedback mechanism, preventing further translocation of NF-κB to the nucleus[9]. The balance between IκB-α destruction and synthesis therefore provides a highly effective feedback control mechanism for regulating NF-κB activity.

Table 1. The major transcription factor families

Family	Examples	Binding form	DNA-binding domain	Structural information[a]
Zinc finger	WT-1, Sp1	Monomer	Four Cys or His coordinated Zn repeated >3 times	X-ray, NMR
Basic coiled-coil (bZIP)	Fos, Jun	Homo-/heterodimer	α helix with heptad of hydrophobic residues	X-ray, NMR
Basic helix–loop–helix	Myc, Max	Homo-/heterodimer	Basic region flanked by α helices	X-ray
Homeodomain	Oct-1, Hox	Monomer	60 amino acids similar to helix–turn–helix	X-ray, NMR
Rel-related	c-Rel, NF-κB	Homo-/heterodimer	300 amino acid Rel domain	X-ray
Nuclear receptor Zn domain	GC, retinoids	Monomer then dimer	Two sets of 4 Cys binding Zn	X-ray, NMR

[a] Reviewed in Ref. 65

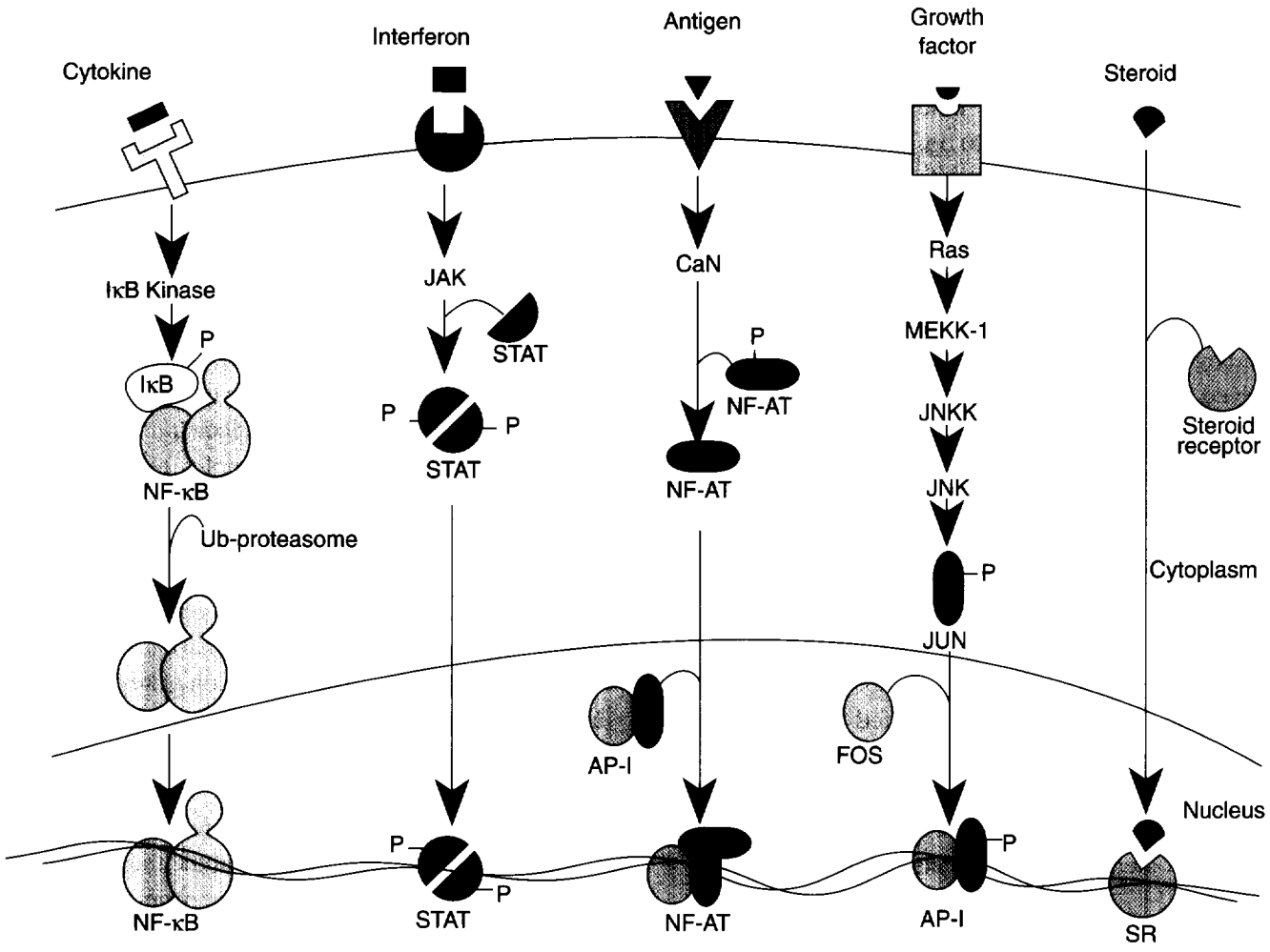

Figure 2. *Examples of the diverse mechanisms that regulate the transcriptional activation potential of transcription factors (TFs). Subcellular localization of NF-κB is regulated by the binding of a cytoplasmic inhibitor, IκB-α. Inducible phosphorylation of IκB-α leads to its destruction by components of the ubiquitin (Ub)–proteasome pathway, facilitating the nuclear translocation of NF-κB. Members of the STAT (signal transducers and activators of transcription) family of TFs are regulated by inducible tyrosine phosphorylation, leading to dimerization and nuclear translocation. Dephosphorylation of NF-AT (nuclear factor of activated T cells) is required for nuclear localization and complex formation with AP-1. Complex kinase pathways, such as those for the inducible phosphorylation of the c-Jun component of AP-1, regulate AP-1-directed transcriptional activation. Direct binding of diffusible ligands, such as steroid hormones, to cytoplasmic receptors can result in nuclear translocation and DNA binding. CaN, calcineurin; JAK, Janus kinase; JNK, Jun N-terminal kinase; JNKK, Jun N-terminal kinase kinase; MEKK-1, MAP kinase kinase kinase-1.*

Regulated phosphorylation

A second, widely utilized, mechanism for TF regulation involves the inducible phosphorylation of key residues that control either subcellular localization or affinity for DNA. Specialized kinases, activated by the binding of extracellular factors to cell surface receptors, mediate these events. Many examples of TFs whose activity is enhanced by the phosphorylation of either serine or tyrosine residues exist, including the c-Jun component of AP-1 and the STAT (signal transducers and activators of transcription) family of TFs. AP-1 is a sequence-specific transcriptional activator composed of members of the Jun and Fos families[10]. These proteins, which belong to the bZIP group of DNA-binding proteins, associate to form a variety of homo- and heterodimers that bind a common enhancer element. The activities of both pre-existing and newly synthesized AP-1 components are modulated through their phosphorylation.

In the case of c-Jun, phosphorylation at a cluster of sites adjacent to its basic region inhibits DNA binding by c-Jun homodimers, but not by c-Jun–c-Fos heterodimers[11]. In contrast, phosphorylation of c-Jun at Ser73 and Ser63, located within its transactivation domain, potentiates its ability to activate transcription as either a homodimer or a heterodimer with c-Fos. These residues, which do not affect DNA binding, are phosphorylated by the newly discovered members of the MAP (mitogen-activated protein) kinase family, the Jun N-terminal kinases (or JNKs)[12]. A novel family of cytoplasmic protein tyrosine kinases, termed the Janus kinases (JAKs), associate with a family of cytokine receptors and are catalytically activated following ligand binding[13]. The activated JAKs phosphorylate tyrosine residues present in the STAT family of TFs, leading to dimerization and nuclear translocation[14].

Dephosphorylation represents another distinct mechanism for regulation of TF activity. Activation and nuclear translocation of NF-AT (nuclear factor of activated T cells) is regulated through the inducible dephosphorylation of complexes found within the cytoplasm, an event apparently mediated by the Ca^{2+}-activated phosphatase calcineurin[15]. Dephosphorylation of NF-AT results in its nuclear translocation, association with AP-1 and binding to the promoters of genes required for T cell activation[16].

Activation by direct ligand binding

The direct binding of soluble ligands to cytoplasmic TFs, resulting in their nuclear translocation and potentiation of DNA binding, represents yet another mechanism for TF regulation. Many hormones, including glucocorticoids (cortisol and aldosterone), estrogen, androgen (dihydrotestosterone), vitamin D (1,25-dihydroxycholecalciferol) and thyroid hormone, modulate gene expression via this mechanism[17]. These hormones bind to specific cytosolic receptors that are all members of the steroid hormone receptor supergene family[18]. Some of these receptors can be nuclear in origin, as in the case of the retinoid receptor gene family[19]. Each of these factors contains, in addition to one or more activation domains, a DNA-binding domain, consisting of two zinc fingers, and a hormone-binding domain. Hormone binding is absolutely required for DNA binding and for the transcriptional activation of hormone-responsive genes.

Role of dimerization

Regulated dimerization of distinct members of a TF family can also differentially regulate their transactivating potential. For example, the c-Myc oncoprotein, which is involved in the progression of a wide range of neoplasias, dimerizes with its partner, Max, to bind DNA, activate transcription, and promote cell proliferation, as well as programmed cell death[20]. Max also forms homodimers or heterodimers with an alternative partner Mad (Figure 3). These complexes behave as antagonists of Myc–Max through competition for common DNA targets[21]. Myc–Max formation is primarily regulated by the level of cellular Myc production, which is itself regulated by other TFs. Enhancement of Myc–Max levels, either through overproduction of Myc, or mutation of Myc resulting in its stabilization, is commonly associated with neoplastic transformation[22].

These represent but a few examples of the diverse mechanisms that regulate TF activity. Additional mechanisms that

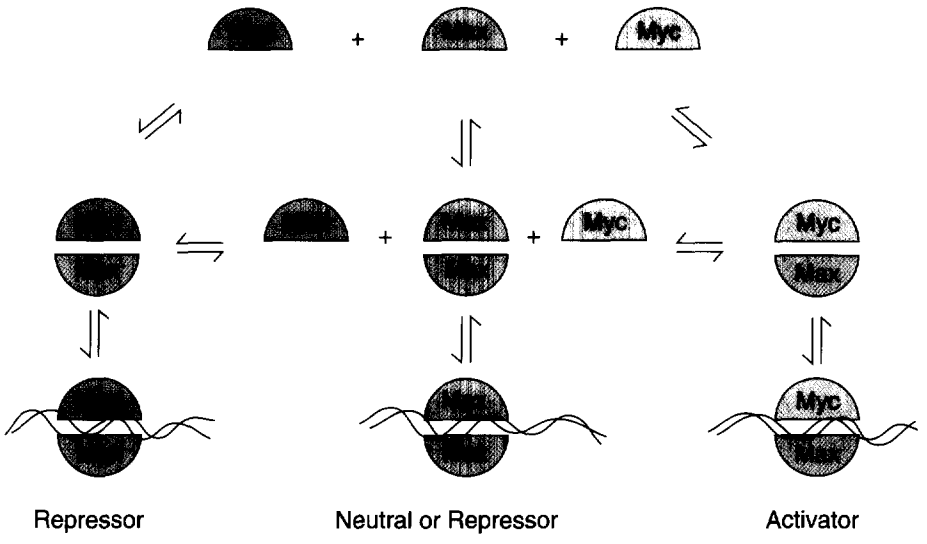

Figure 3. *Differential dimerization of Myc, Max and Mad can have dramatically different effects on transcriptional activity. Elevated expression of Myc leads to an increase in Myc–Max heterodimers, and is associated with uncontrolled cellular proliferation. In the absence of Myc, Max–Max homodimers and Max–Mad heterodimers repress transcription from a variety of growth-regulatory genes.*

regulate either the affinity of TFs for DNA or the potential for transactivation have been reported, with many acting within the nucleus as well as in the cytoplasm. Perturbation of any of these mechanisms can lead to aberrant TF activity and disease.

Role of transcription factors in human disease

Alterations in the activity of specific TFs form the basis of many human diseases. Our understanding of the role of TFs in different diseases has been formed by two routes. The first represents studies of therapeutics whose molecular mechanism of action is unknown. These studies represent a link to the past, where random screening successfully identified drugs whose mechanism of action was unknown. With the advent of molecular biology, it became possible to elucidate the mechanism of action of many such agents, motivated primarily by the hope that such an understanding could enhance the efficacy and reduce the side effects of subsequent generations of these drugs.

The second approach represents an empirical definition of the pathologic role of TFs through knowledge of their key role in the regulation of disease-related genes. Together, these two approaches have identified TFs that appear to play key roles in the progression of human disease, thereby focusing significant attention upon these TFs as targets for drug discovery.

NF-AT and immune disease

Because of their suppressive effects on the immune system, cyclosporin A (CsA) and FK506 are widely used to prevent graft rejection following transplantation[23]. CsA and FK506 exert their immunosuppressive potential by blocking T cell receptor-mediated transcription of the interleukin 2 (IL-2) gene and other genes important for T cell activation[24]. CsA and FK506 bind intracellular proteins belonging to the immunophilin family and these complexes inhibit the cytoplasmic Ca^{2+}-binding phosphatase calcineurin[25]. This inhibition prevents the dephosphorylation and nuclear translocation of NF-AT (see Figure 2). The correct DNA binding of NF-AT to regulatory elements in the IL-2 promoter contributes to the transcriptional activation of this gene. Clinical use of CsA and FK506 is limited by their significant side effects, which are likely to be caused by the pleiotropic metabolic effects these agents exert through binding to immunophilins. The identification of NF-AT as a molecular target in T cell activation suggests a more direct, molecular-based approach to the development of immunosuppressive agents with the potential for improved efficacy and reduced side effects.

TFs and cancer

Modulation of TF activity can alter the expression of growth-regulatory proteins. It is not surprising, therefore, that multiple TFs are found as oncogenes or tumor suppressor genes. Altered expression or function of several TFs is found in a wide variety of tumors (e.g. c-Myc in lung, breast and cervical carcinoma[26], p53 in most known tumors[27]), whereas alterations in others is more limited to specific neoplasms (e.g. WT-1 in Wilms' tumor[28]). Several TFs have been identified as playing a role in the regulation of normal cellular proliferation and senesence, and pharmacologic modulation of these factors has been suggested to offer potential for new cancer treatments[29].

DNA has long been recognized as the target for carcinogens and antitumor agents. Recent evidence indicates that certain of these agents can form specific DNA adducts, which alter transcriptional activity in the nucleus[30]. In some cases, these DNA-reactive molecules and their DNA adducts have been shown to bind TFs, or to act as surrogate TFs themselves. Other agents can act to antagonize TF function or to influence TF activity through specific interaction of the TF with the altered DNA adduct. For example, cisplatin, a drug approved for the treatment of testicular and ovarian cancer, binds DNA and in so doing creates binding sites for human upstream binding factor (hUBF), an activator of ribosomal RNA transcription[31]. hUBF is one member of a family of proteins, which includes the high-mobility group factors, and displays a high affinity for bent DNA structures.

Another agent, (+)-CC-1065, one of a series of novel drugs in clinical trials for several forms of cancer, has been shown to act as a surrogate for the TF Sp1 (Ref. 32). This drug targets specific residues in the minor groove of DNA, with one such site being present in the DNA between the GC boxes in the SV40 tumor virus early promotor. This region represents an important locus for Sp1-induced bending and looping of the DNA, an effect believed to promote protein–protein interactions which increase transcription. (+)-CC-1065 binding entraps and intensifies the bending of DNA in this region, effectively substituting for Sp1 and possibly accounting for its modulation of transcription. Such insights into the mechanisms by which DNA-reactive agents can modulate transcription will certainly influence the future development of chemotherapeutic agents and might also suggest possibile routes for the synthesis of TF mimetics.

TFs and cardiovascular disease

The cholesterol-lowering agent mevastatin and its analogs have received significant attention recently because of their

demonstrated efficacy in the prevention of coronary heart disease[33]. Cholesterol balance in mammalian cells is maintained in part by sterol-mediated repression of gene transcription for the low-density lipoprotein receptor (LDLR), a cell-surface transmembrane protein[34]. In hypercholesterolemia, reduced levels of LDLR increase the risk for myocardial infarction and death[35]. An LDLR promoter sequence, termed the sterol response element (SRE), is essential for the cholesterol-mediated repression of LDLR transcription and is recognized by a zinc-finger-type TF called CNBP-SREBP[36]. Pharmacologic reduction of intracellular cholesterol levels results in enhanced transcription of the LDLR gene[37]. Direct inhibition of CNBP-SREBP may provide additional opportunities in the pharmacologic modulation of blood lipids.

Atherosclerosis is the most significant vascular disorder leading to serious morbidity or death in the Western world[38]. It is now accepted that the initial steps in the formation of the atherosclerotic lesion involve the adherence of circulating monocytes to dysfunctional vascular endothelium and transmigration into the lumen. Once in the subendothelial space, monocytes transform into macrophages, take up substantial amounts of lipids and become foam cells. Localized production of growth factors and cytokines by foamy macrophages (and other cells) contributes to the development of atherosclerotic lesions. TFs present in vascular endothelium, in particular NF-κB and AP-1, have been shown to regulate most of the genes involved in the initiation of the atherosclerotic lesion and as such may represent targets for the development of antiatherosclerotic agents[39].

TFs and infectious diseases

Viral replication in mammalian cells involves a complex series of events regulated at both the transcriptional and post-transcriptional levels. Virally encoded TFs represent attractive targets for drug design because they are expressed only in infected cells and do not appear to have functional counterparts in non-infected cells.

The herpes simplex virus transactivator VP16 directs the assembly of a multicomponent protein–DNA complex with cellular TFs Oct-1 and VCAF-1, contributing a potent C-terminal acidic activation domain that is essential for viral replication in mammalian cells[40]. Human papillomavirus (HPV) infection is causally associated with benign and malignant neoplasia of mucosal and cutaneous epithelia. E2, a virally encoded TF, regulates the transcription of other viral proteins involved in modulation of host cell TF activity[41]. Disruption of normal E2 activity is associated with HPV types 16 and 18, and is associated with high risk for genital carcinogenesis[42]. Pharmacologic antagonists of VP16 and E2 function may represent novel antiviral agents. Cellular TFs such as NF-κB and Sp1 have been shown to be required for transcription of the human immunodeficiency virus (HIV), and as such have been suggested as targets for inhibition of HIV replication[43].

NF-κB and inflammatory diseases

Pharmacologic modulation of leukocyte–endothelial adhesion has become a focus for the development of novel anti-inflammatory agents. Attenuation of granulocyte-mediated tissue injury would provide new therapeutic opportunities in a variety of acute inflammatory disorders, including hemorrhagic and septic shock, allograft rejection, ischemia–reperfusion injury, acute airway inflammation and the pulmonary complications associated with cardiopulmonary bypass[44]. Such agents may also hold promise in the treatment of chronic inflammatory diseases such as asthma, arthritis and atherosclerosis. Leukocyte recruitment to sites of inflammation is regulated by the elevated expression of endothelial cell adhesion molecules and proinflammatory cytokines. Examination of the promoters of these genes revealed that a single TF, NF-κB, plays a critical role in their transcriptional activation[45].

Pharmacologic inhibition of NF-κB in cultured endothelium results in the simultaneous and selective inhibition of the adhesion molecules E-selectin, VCAM-1 and ICAM-1 (Refs 46, 47). NF-κB activation in animal models of acute inflammation precedes the elevation in inflammatory gene expression and leukocyte recruitment[48]. NF-κB also regulates many proinflammatory and prothrombotic factors produced by activated leukocytes[49]. NF-κB represents a master regulator of inflammation and is, therefore, an attractive target for drug development[50].

Further support for NF-κB as a molecular target emerged from studies of the mechanism of action of glucocorticoids, which have been used for decades as clinical tools to suppress both the immune response and the process of inflammation. The molecular mechanisms which underlie their therapeutic effects are poorly understood. Glucocorticoids bind to a cytoplasmic glucocorticoid receptor, a member of the steroid hormone receptor superfamily, promoting nuclear translocation and DNA binding (see Figure 2). Cell adhesion molecule and cytokine gene expression is repressed by glucocorticoids even though these genes lack glucocorticoid responsive elements. Glucocorticoids inhibit activation of NF-κB both *in vitro* and *in vivo* through transcriptional activation of the IκB-α gene[51]. Glucocorticoid inhibition of NF-κB activation

could account for its anti-inflammatory properties. Inhibition of NF-κB, therefore, represents a novel approach to anti-inflammatory drug development which could provide agents with the efficacy of glucocorticoids but without their hormonal side effects.

Future prospects

The above examples represent but a few of the emerging TF targets for new drug discovery. They serve to demonstrate the diversity of pathologic processes regulated by TFs and suggest, therefore, that TF therapeutics will have broad applications in the treatment of disease. The examples also demonstrate how different approaches have been successfully applied to the definition of the role of TFs in disease. With the discovery of new TFs and the relentless progress of molecular medicine, these are likely to represent only several of many new molecular targets for drug discovery.

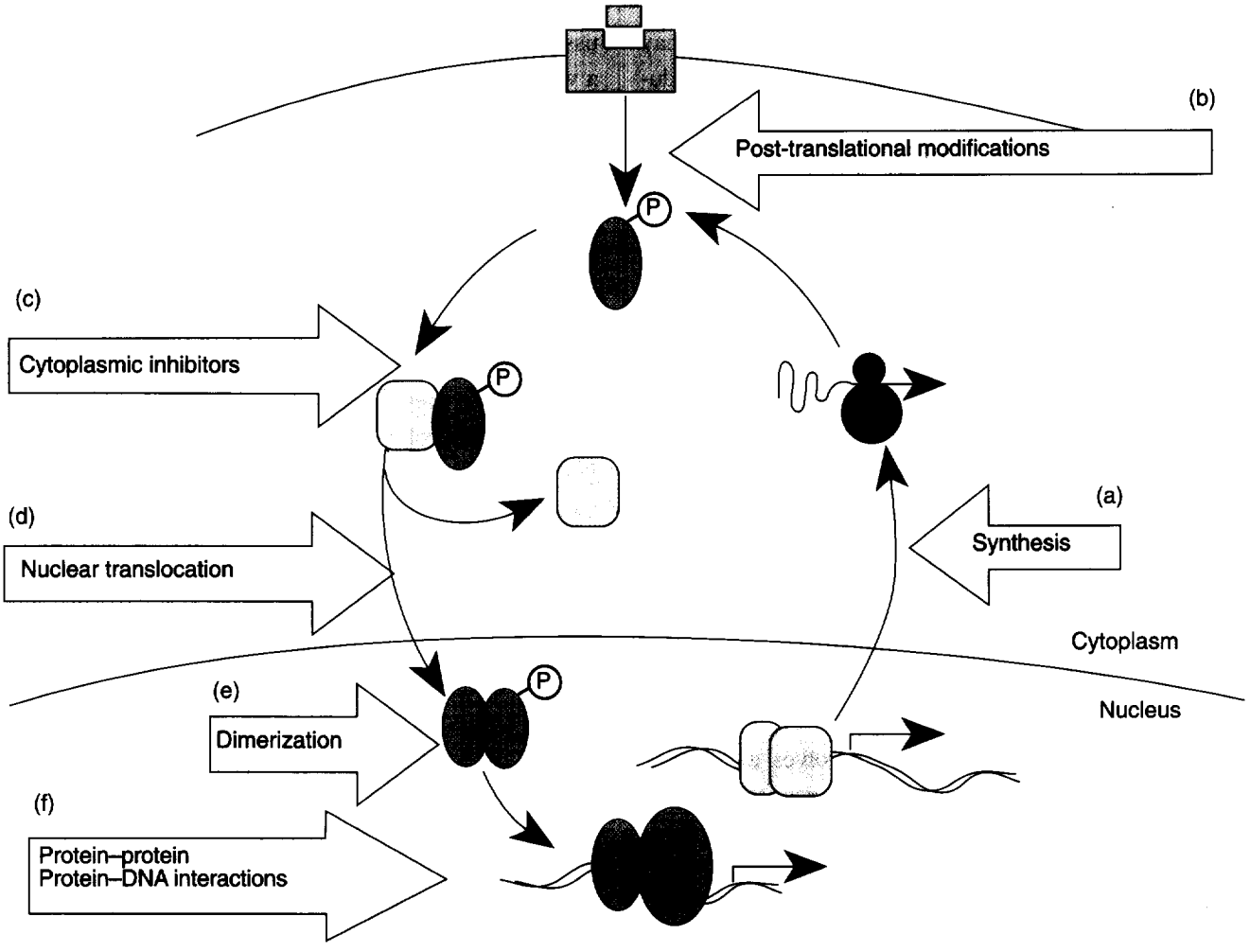

Figure 4. *Potential strategies for transcription factor (TF) drug development. An understanding of the mechanisms regulating TF activity suggests that different strategies to modulate either the production or the transactivating potential of any TF (see text). (a) Antisense oligonucleotides have been developed to block TF synthesis. (b) Inhibitors of signal transducing enzymes such as kinases and phosphatases can inhibit key post-translational modifications which affect TF function. (c) Inhibition of the destruction or augmentation of the production of cytoplasmic inhibitors can deny TFs access to the nucleus and DNA. (d) Inhibitors of mechanisms by which TFs translocate to the nucleus would have a similar effect. (e) Inhibitors of TF dimerization or (f) protein–protein or protein–DNA interaction would not affect post-translational modification or nuclear translocation of a TF, but would inhibit its transactivating potential.*

Strategies for TF drug development

Increased understanding of the regulation of TF activity suggests rational approaches to drug development, as reviewed here and elsewhere[52,64]. Each of the steps in the life cycle of a TF represents a potential point of therapeutic intervention (Figure 4). Careful selection of the strategy for TF modulation is required to maximize the chances of successful drug development.

TF synthesis

Antisense RNAs that bind and destabilize the mRNA for TFs have been pursued as potential therapeutics (Figure 4, a). Introduction of short synthetic RNA molecules of opposite polarity to the mRNA encoding a TF have been successfully used to inhibit production of many TFs in cultured cells and *in vivo*. Antisense RNA against the TF c-Myb has been used successfully to treat human leukemia in a mouse model[53], and to prevent restenosis following balloon angioplasty in rats[54]. Antisense inhibition of NF-κB prevents tumor metastasis and actually promotes regression of human tumors in mice[55]. Several major challenges face the development of antisense therapeutics, however, including effective delivery of these macromolecules to the cytoplasm of cells in different organs and the demonstration of efficacy and safety in human clinical trials. Answers to these questions should emerge from several trials now under way[56].

Post-translational modifications

As most TFs require post-translational modifications such as phosphorylation or dephosphorylation for activation, kinases and phosphatases involved in signal transduction are obvious drug targets (Figure 4, b). Protein kinases represent the largest known protein family, with over 175 different members having been identified to date[57]. These proteins share a homologous 'kinase' domain which consists of 250–300 amino acid residues. Because of the structural and functional homology among kinase domains, the identification of specific inhibitors represents a significant challenge for drug discovery. However, specific inhibitors of several different MAP kinases have been reported[58,59], and these are likely to represent only the first of many such agents.

Many signaling proteins can regulate the activity of multiple TFs and other cellular proteins. Definition of the JAKs and JNKs as specific for activation of a finite number of related TFs suggests that selective inhibition of one or more TFs can be achieved[12,13]. These agents would affect the expression of multiple disease-related genes, and could therefore represent highly efficacious therapeutics. Elucidation of the three-dimensional structure of several kinases and phosphatases has made possible the application of structure-based drug design to the development of selective kinase inhibitors[60].

Cytoplasmic inhibitors

Manipulation of the levels of TF cytoplasmic inhibitors represents another approach to drug development (Figure 4, b). The activation of NF-κB is achieved through the destruction of IκB-α, mediated by sequential phosphorylation, ubiquitination and proteolysis by the 26S proteasome. Pharmacologic inhibition of any of these three processes results in inhibition of NF-κB activation[46,47]. Somatic gene therapy may also provide the opportunity to augment the expression of genes encoding TF inhibitors. The use of tissue-specific and drug-inducible promoters may ensure appropriate expression in target tissues only[61].

Nuclear translocation

The process of controlled nuclear translocation of activated TFs may provide another approach to drug discovery (Figure 4, c). The process of nuclear import of proteins is poorly understood[62], and it is unclear whether inhibition of a specific protein could result in an agent which selectively inhibits TF translocation but not nuclear import of other essential proteins. However, novel double-stranded oligonucleotides encoding the DNA-binding sites for select TFs, when introduced into the cytoplasm of cells in culture, can act as TF 'decoys', binding activated TFs and retaining them within the cytoplasm[63]. These agents may represent a novel application of synthetic oligonucleotides as inhibitors of TF nuclear translocation.

Dimerization, protein–protein and protein–DNA interactions

Many TFs belong to families which must dimerize to effectively bind DNA (Figure 4, d). In addition, once bound to DNA, TFs must effectively interact with other TFs and components of the basal transcription complex to modulate RNA Pol II activity (Figure 4, e). Pharmacologic antagonism of protein–protein or protein–DNA interactions within the nucleus represents attractive possibilities for drug discovery[64].

The three-dimensional structures of several TFs bound to DNA as either monomers or dimers have been reported[65–68], providing significant information upon which to initiate structure-based drug design efforts. This structural information revealed that TFs dimerize and interact with DNA through

distinct mechanisms, suggesting that specific inhibitors of these processes could be developed. For example, inhibitors of Myc–Max dimerization that have no effect on Mad–Max heterodimerization would result in inhibition of Myc-induced gene expression (see Figure 3), and might have application in the treatment of proliferative diseases such as cancer or restenosis[20]. In addition, new technologies such as plasma resonance and scintillation proximity[69], which measure protein–protein interactions with hitherto unrealized sensitivity, have facilitated the development of assays with which to screen large numbers of chemical entities for drug leads.

Conclusion

The past several years have seen an explosion in our understanding of the role of TFs in human disease and the definition of a number of TFs as potential molecular targets for drug discovery. At the same time, our knowledge of the structure, function and mode of regulation of these TFs has also significantly increased. These advances have set the scene for drug discovery efforts which are just beginning to bear fruit.

In the next few years, new classes of chemical entities should be described which inhibit TF activity and which are candidates for clinical development. Significant challenges face the development of TF drugs, not the least of which is the need to target only those TFs that regulate the limited subset of aberrantly expressed genes in specific cells implicated in disease. Such specificity will be required if we are to realize the potential of TF drugs as efficacious therapies with minimal side effects. In addition, well-designed and comprehensive human clinical trials will be required to prove the concept that pharmacologic modulation of TFs truly represents a novel approach to disease modification. If recent progress is anything to go by, these issues will be answered within the near future.

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