Review

Transcription Factors and Neoplasia: Vistas in Novel Drug Design

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Abstract

Purpose: **The fundamental role of gene transcription and the recognition of transcription factors as important control elements of cell growth, differentiation, and programmed cell death (apoptosis) aroused an ever-increasing interest for these proteins as potential pharmaceutical targets for therapeutic intervention in various diseases, among them cancer.**

Experimental Design–Results: **The vast array of information available for their molecular architecture and mode of action in various biological contexts, combined with the new opportunities offered by the flourishing technologies of structure-based drug design, computer-aided modeling, and functional genomics/proteomics, are creating an exciting scenery for the development of a novel generation of highly selective drugs.**

Conclusions: **This transcription factor-based therapeutic approach may revolutionize the anticancer drug options and will add significantly to the current clinical armamentarium.**

Introduction

Regulation of gene expression is a prominent component of a complex network that dictates normal development and proper function of the organism. Gene regulation is primarily achieved at the level of gene transcription, whereby the genetic information inherited in DNA is copied into an RNA transcript. Different genes are being transcribed in different cell types and in response to various stimuli, such as hormones, growth factors, changes in nutritional state, stress, injury, and inflammation, resulting in control of cell cycle progression, cellular proliferation, differentiation and cell shape, mobility, and interactions with neighboring cells.

A pivotal role in the gene transcription process has a class of key proteins, known as transcription factors. The enormous advances in genetic engineering techniques over the last decade have made possible their identification and elucidation of their structure and the signaling pathways that modulate their function. Aberrations in their biochemical properties or the regulatory mechanisms that fine-tune their activity, because of either genetic defects or abnormal internal/external cues, can lead to a variety of clinical entities, from developmental disorders and diseases of the hemopoietic, immune, and endocrine system to several malignancies.

It is noteworthy that the main targets of >10% of the 50 best selling drugs approved by the United States Food and Drug Administration are the transcriptional regulatory circuits (1). Paradigms of transcription-based drugs are the immunosuppressant cyclosporin A, the anti-inflammatory salicylates, thiazolidinediones for type II diabetes mellitus, and tamoxifen/raloxifene for the treatment of breast cancer (2, 3). Although the use of drugs that influence transcription factor function increases continuously, none of them has been identified on the basis of its ability to target transcription. Typically, most of these pharmaceuticals were isolated in screens designed to select for specific biological effects rather than by selecting for compounds that directly target gene transcription (4). During the past few years, structure-based ligand design (*i.e.*, the search for small molecules that fit into the binding pocket of a given target and can form favorable interactions) has gained an increasingly prominent position within the drug discovery process (5). An impressive number of convincing examples have been published that prove the potential of this approach, and a number of drugs that have been designed by this procedure are now in clinical trials (6).

Focusing on cancer, mutations, and deregulated functioning of transcription factors encoded by (or related to) certain proto-oncogenes and tumor suppressor genes are crucially involved in various types of malignant transformation. The discovery of small molecule compounds aiming at particular transcriptional targets that participate in the development and progression of the cancer phenotype represents the future perspective in substituting the classic cytotoxic and hormonal anticancer agents of the past by more selective drugs, with greater efficacy and minimal side effects.

The Transcriptional Machinery: An Overview

Transcription of protein-coding genes in eukaryotic cells is regulated by an ensemble of proteins, whose central component is the enzyme RNA polymerase II (pol II). To accomplish its action efficiently, pol II requires a battery of accessory proteins termed transcription factors. The critical importance of these proteins for cell physiology is illustrated by the observation that mutations in their encoding genes result in a wide gamut of human pathologies, from developmental anomalies to cancer (7).

Transcription factors are divided into two major groups (8).

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BTFs[2] are DNA- or non-DNA-binding proteins, essential for the transcription of all protein-coding genes. Their main duty is to recruit pol II at the core promoter region of the gene. This region contains characteristic regulatory sequences, such as the TATA box (situated 25–30 bp upstream from the transcription start site) or the initiator element (overlapping the transcription start site). BTFs are recognized by these sequences, around which a complex incorporating pol II is formed in a step-wise fashion (9).

GSTFs are required only for a subset of genes transcribed by pol II. They recognize specific sequence elements, typically, 6–12-bp-long, located downstream or upstream within few hundred bp (promoters) or several kbp (enhancers) from the transcription start site. These factors interact with any promoter or enhancer that contains the cognate binding site and affect the basal transcriptional machinery, regulating the transcription initiation rate, hence, expression level of the corresponding gene. Their ultimate action may be either positive (activators) or negative (repressors).

Various potential mechanisms exist whereby DNA, GSTFs, and the BTA (pol II and BTFs) are brought together to form an active transcription complex (10). Interactions of GSTFs with other proteins are of principal importance for the regulation of their activity and the mechanism of their action, *e.g.*, several GSTFs interact with the basal transcription complex through mediator factors (coactivators/corepressors), which are non-DNA-binding proteins that can also be specific to certain GSTFs or promoters/enhancers (11). Moreover, "cross-talk" between DNA-bound GSTFs (*i.e.*, competition for common limiting cofactors) may have synergistic or antagonistic effects on the rate of transcription.

Physiological or pathological stimuli that are transmitted to the nucleus trigger/destruct the cooperative action of all these factors, influencing cell growth, differentiation, and homeostasis.

Molecular Anatomy of GSTFs

GSTFs achieve their regulatory function by virtue of their modular architecture. These factors have two distinct activities: (*a*) they bind specifically to their DNA-binding site; and (*b*) they activate (or repress) transcription. These activities can be assigned to separate protein domains called DNA-binding domains and transeffecting domains, respectively. In addition, many GSTFs occur as homo or heterodimers, held together by dimerization domains. A few GSTFs have ligand-binding domains that allow regulation of transcription factor activity by binding of an accessory small molecule (*e.g.*, hormone). The steroid hormone receptors (see below) are an example containing all four of these types of domain.

Typically, a DNA-binding domain has a helical shape (α-helix) within or adjacent to which are nested positively charged (basic) amino acids. On the basis of the presence of highly conserved phylogenetic motifs in DNA-binding/dimerization domains, GSTFs can be categorized into four major families that include >80% of the known GSTFs (Fig. 1; Refs. 8, 12, and 13).

The HTH motif is characteristic of DNA-binding proteins containing a 60-amino acid homeodomain, which is encoded by a sequence called the homeobox. The motif comprises three consecutive α-helices, the third of which interacts with the DNA. Many homeodomain-containing proteins bear additional motifs that are closely related to the HTH motif and regulate indirectly to the DNA-binding affinity of the transcription factor and the specificity of the recognized DNA sequences (14). An example of these combined motifs are the POU proteins (*e.g.*, Pit-1 and Oct-1/2), which play an important role in the regulation of gene expression during embryonic development. Intense clinical interest has been generated for the HTH transcription factors, because some of them have been implicated in the development of acute lymphoid leukemia in children, as well as in other neoplasms (15).

The zinc finger motif has a loop of 12 amino acids anchored by two cysteine and two histidine (alternatively, four cysteine) residues that tetrahedrally coordinate a zinc ion. This motif folds into a compact structure that can be repeated several times. Most zinc finger transcription factors bind to DNA as dimeric proteins. A special class of ligand-activated zinc finger proteins is the superfamily of NRs, which includes the receptors for steroid/thyroid hormones, retinoids, vitamin D_3, and certain fatty acids (16, 17). Several studies revealed that many of these receptors are involved in the development of various cancers, through activation of the normally hormone-regulated genes in a hormone-independent manner. Other zinc finger transcription factors implicated in carcinogenesis are the tumor suppressor gene product WT1, which is inactivated in children with WT, and the proto-oncogene product bcl-6, which has an important role in large cell lymphoma.

LZip proteins contain a hydrophobic leucine residue at every seventh position in a region that is often at the COOH-terminal part of the DNA-binding domain. These leucines lie in an α-helical region, and the regular repeat of these residues forms a hydrophobic surface on one side of the α-helix with a leucine every second turn of the helix (Fig. 1). These leucines are responsible for homo/heterodimerization through interactions between the hydrophobic faces of the α-helices. This interaction forms a stable coiled-coil structure. The LZip motif (also used as a dimerization domain in some homeodomain-containing proteins) is of special interest, as it underlies the interactions between the products of the *jun*- and *fos*-family proto-oncogenes. These proteins form dimers, known as AP-1 transcription factors, which are nuclear effectors of various signal transduction cascades and are thought to play key roles in cell proliferation, differentiation, apoptosis, and oncogenic cell transformation (18, 19).

The overall structure of the HLH motif is similar to the LZip, except that a nonhelical loop of polypeptide chain sepa-

[2] The abbreviations used are: BTF, basal transcription factor; GSTF, gene-specific transcription factor; BTA, basal transcriptional apparatus; CBP, (c-AMP response element binding)-binding protein; HTH, helix-turn-helix; NR, nuclear receptor; WT, Wilm's tumor; LZip, leucine zipper; AP, activator protein; HLH, helix-loop-helix; HAT, histone acetyltransferase; RB, retinoblastoma; HDAC, histone deacetylase; AF, activation function; RAR, retinoic acid receptor; NF-κB, nuclear factor-κB; CDK, cyclin-dependent kinase; PML, promyelocytic leukemia; t-RA, all-*trans* retinoic acid; ER, estrogen receptor; TGF, transforming growth factor; IGF, insulin-like growth factor; siRNA, short interfering RNA.

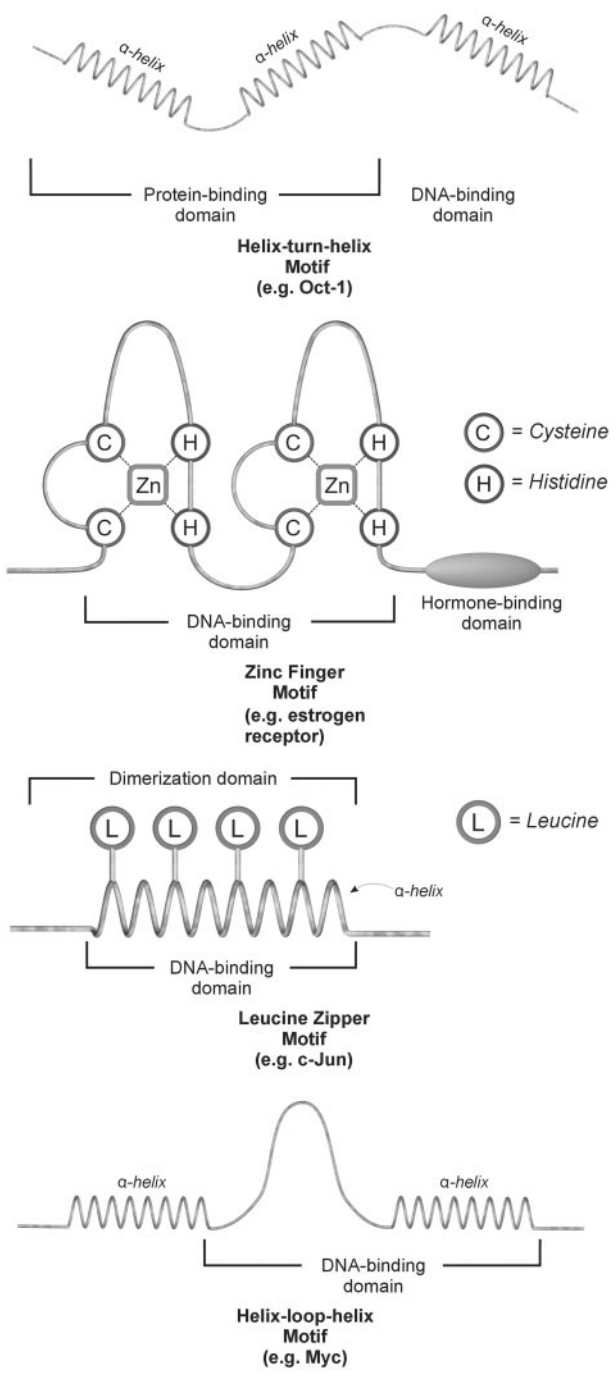

Fig. 1 Classification of GSTFs. The general structure of each of the major classes of GSTFs is depicted. The types of GSTFs are named after the characteristic motifs involved in DNA binding and protein dimerization. Each structure is illustrated with an example.

rates two α-helices in each monomeric protein. Hydrophobic residues on one side of the COOH-terminal α-helix allow homo/heterodimerization. This structure is found in the MyoD family of proteins (which perform key functions in a regulatory pathway that determines the identity of myoblasts and controls their differentiation) and the products of the *myc* proto-oncogenes. As with the LZip, the HLH motif is often adjacent to an NH_2-terminal region rich in basic amino acids that requires dimerization for DNA binding. With both HLH and LZip proteins, the formation of heterodimers allows much greater diversity and complexity in the transcription factor repertoire.

Transeffecting domains (transactivation/transrepression domains) mediate the interaction of GSTFs with components of the BTA and/or with other transcriptional modulators (*e.g.*, coregulators). Transactivation domains can stimulate transcription and include acidic activation domains (containing a high proportion of negatively charged amino acids, *e.g.*, glucocorticoid receptor), glutamine-rich domains (*e.g.*, transcription factor Sp1), and proline-rich domains (*e.g.*, c-Jun, AP-2, and Oct-2). Transrepression domains are responsible for direct inhibition of transcription, *e.g.*, a domain of the thyroid hormone receptor can repress transcription in the absence of thyroid hormone and activates transcription when bound to its ligand. Similarly, WT1 has a specific proline-rich repressor domain that lacks charged residues. Transeffecting domains are characterized generally by structural "plasticity" that allows the GSTF to participate in multiple protein-protein interactions (20).

Finally, most GSTFs harbor additional regions that also regulate their functions. These include nuclear localization sequences, sites for post-translational modification, and sequences recognized by specific degradation systems.

Multistage Regulation of Transcription Factors

The activity of GSTFs can be regulated at the level of synthesis, which is commonly encountered in developmental regulators. In contrast, physiological regulators are often controlled at the protein level, allowing for a more rapid response (21). GSTFs are induced in response to a plethora of external stimuli, which provoke the activation of various signal transduction pathways to transmit the signal from the extracellular environment to the nucleus. The majority of the components comprising these pathways are kinase and phosphatase enzymes that regulate the phosphorylation state of several intracellular substrates, ultimately resulting in the serine/threonine and, in certain cases, tyrosine phosphorylation or dephosphorylation of GSTFs. These reversible post-translational modifications are used as a means to modulate several aspects of GSTF function, including DNA binding, transactivation/transrepression, dimerization, subcellular localization, and protein stability (22, 23). The nature of these biochemical alterations is of significant importance when considering new drug development.

Reversible acetylation is an additional means to controlling transcriptional responses, with an ever-increasing importance. To date, the focus has been on modification of the histone components of chromatin (24); however, a growing number of GSTF targets has been recognized (25). Histone acetylation (*i.e.*, the attachment of acetyl groups to lysine amino acids in the NH_2-terminal regions of each of the core histones) is catalyzed in the nucleus by HAT type A and correlates with transcription activation. A variety of coactivators have HAT A activity, which may allow them to loosen the association of nucleosomes with a gene's control region, and possibly also to reduce the interaction between individual nucleosomes that leads to formation of the 30-nm chromatin fiber, thereby enhancing transcrip-

tion (26), *e.g.,* a mammalian coactivator called p300/CBP, which had been ascribed a clearly defined role in activation of a variety of genes (see below), was found to be a HAT, thus functioning, at least in part, by affecting nucleosome positioning rather than by influencing assembly of the preinitiation complex (27). On the other hand, transcriptional repressors, such as the *RB1* gene product (see below), bind to DNA sites and interact with corepressors, which in turn bind to HDACs, *i.e.,* enzymes that remove acetyl groups from core histones; Ref. 28). This assembly of ternary protein complexes brings the HDACs close to nucleosomes in the neighborhood. The deacetylation of core histones allows the basic tails of the histones to bind strongly to DNA, stabilizing the nucleosome, and inhibiting transcription. Notably, phosphorylation of GSTFs often affects their ability to recruit complexes containing either acetyltransferases or deacetylases. It is plausible that cascades might also exist where acetylation is used as the switch that sequentially turns on downstream components, and/or phosphorylation directly affects the activity of an acetyltransferase/deacetylase (or *vice versa*). Given that chromatin and chromosomes represent the true environment for transcriptional control, answers as to whether histone/GSTF acetylation plays a wider role in signaling and how far the interplay between phosphorylation and acetylation extends will permit a complete description of specificity-determining mechanisms in nuclear responses to extracellular stimuli.

Several transcription factors stimulate gene expression when activated from preexisting inactive forms after ligand binding. NRs are ligand-dependent GSTFs that regulate decisive events during development, control cellular homeostasis, and induce or inhibit cellular proliferation, differentiation, and apoptosis. Lipophilic hormones, such as steroids/retinoids/vitamin D_3, are able to diffuse through the plasma membrane and are potent ligands for these receptors (29). About 70 NRs have been identified to date, and with some notable exceptions, all members display an identical structural organization comprising a variable NH_2-terminal domain (A/B), a well-conserved DNA-binding domain, crucial for recognition of specific DNA sequences, a linker region with central role in protein-protein interactions with transcriptional coregulators, and a COOH-terminal ligand-binding domain (Fig. 1). Two physically separate transactivation functions, a ligand-independent AF-1 mapping in region A/B and a ligand-dependent AF-2 mapping in the vicinity of the ligand-binding domain, may cooperate in a cell- and promoter-specific manner to elicit the transcriptional activity of NRs (30, 31). Hormone binding to the ligand-binding domain leads to receptor activation through conformational changes; the latter unmask the AF-1/2 domains, which mediate the transcriptional responses. NRs bind as homodimers (*e.g.,* steroid receptors) and/or heterodimers (*e.g.,* RAR, thyroid hormone receptor, peroxisome proliferator-activated receptor, vitamin D receptor), along with the promiscuous heterodimerization partner retinoid X receptor, to stretches of DNA, termed hormone-response elements, and regulate transcription of target genes. Some NRs can also cross-talk with other signaling pathways, resulting in a positive or negative interference with the transeffecting potential of other GSTFs, such as AP-1 and NF-κB (32). Furthermore, other signaling pathways (*e.g.,* mitogen-activated protein kinase and CDK cascades) can target NRs directly and alter their activity (33, 34).

Regulation of GSTF activity is also mediated by specific protein-protein interactions, *e.g.,* the HLH group of proteins are regulated by an inhibitor called Id that lacks a complete DNA-binding domain but contains the COOH-terminal α-helix responsible for dimerization (Fig. 1). Therefore, Id protein can bind to MyoD and related proteins, but the resulting heterodimers cannot bind DNA and, hence, cannot regulate transcription. Other proteins may stabilize the DNA-bound form, change the specificity of the target recognition sequence, enhance degradation, or allow migration of GSTFs to a different subcellular compartment (21).

Our increasing knowledge of the regulatory mechanisms governing the activity of GSTFs and their interactions within an active transcription complex offers a plethora of choices for the development of new drugs with selective molecular targets in different cell types or in different tissues.

Transcription Factors in Oncogenesis

Transcriptional regulation can profoundly affect the course of growth-related diseases, such as cancer. Transcription factor mutations and perturbations in signal transduction pathways modulating their activity contribute significantly to a wide gamut of human malignancies. The spectrum of GSTFs that might be suitable targets for therapeutic intervention is broad, ranging from those with an "inherent" oncogenic potential to those mediating aberrant expression of proteins known to contribute to the malignant properties of cancer cells or failure of classical anticancer therapy. The in-depth understanding of the transcriptional networks underlying tumorigenesis is of vital importance for the designing of new anticancer drugs (4).

I. Cellular Oncogenes. It is now known that about one-third of the so-far identified cellular oncogenes code for nuclear DNA-binding proteins that act as GSTFs.

The *myc*-family genes (c-*myc*, L-*myc*, and N-*myc*) are proto-oncogenes that are activated in a variety of neoplasms, as a consequence of chromosomal translocation or DNA amplification (*e.g.,* N-*myc* in neuroblastomas and c-*myc*/L-*myc*/N-*myc* in lung cancers; Refs. 35 and 36). Under normal circumstances, the expression of *myc*-family genes is controlled tightly by signaling pathways that are triggered by growth factor stimulation. However, in tumors, *myc* expression can be uncoupled from normal mitogenic regulators, leading to high levels of the Myc protein. The latter dimerizes inefficiently and does not appear to form homodimers under physiological conditions. Max is a small protein that dimerizes with all three members of the Myc family. The Myc-Max heterodimers stimulate the transcription of genes required for cell division (37).

The *myb*-encoded protein, which has an unusual DNA-binding domain characterized by regularly repeated tryptophan residues, binds DNA as a monomer and regulates the expression of genes engaged in cell proliferation and DNA synthesis (38). The *ets* family genes code for a number of GSTFs with DNA-binding domains similar to that of Myb. Ets-1 and Ets-2 are activated by growth factors and phorbol esters and can cooperate with AP-1 to stimulate transcription of some genes (39).

Elk-1 plays a central role in cell proliferation by regulating expression of c-*fos* in growth factor-induced cells.

Activation of "oncogenic" transcription factors by translocation of the coding sequence from its normal site on a chromosome to a site on another chromosome, which places it under the control of an active promoter, is a frequent event, especially in lymphomas and leukemias. Oncogenes that are activated by this mechanism include c-*myc* in Burkitt's lymphoma, *bcl-6* in large cell lymphoma, *tal-1* in acute lymphoblastic leukemia, *erg* in myeloid leukemia, *bcl-3* in some B-cell chronic leukemias, *hox-11* in cases of acute childhood T-cell leukemia, and others (40).

In other cases, chromosomal translocations generate "chimeric" proteins that contain functional domains derived from two different transcription factor proto-oncogenes. *E2A* encodes an HLH transcription factor, which is fused to the homeodomain-containing Pbx-1 transcription factor in 20–25% of children with pre-B-cell acute lymphoblastic leukemia; *fli-1*, a member of the *ets*-gene family, is fused to the transcription factor-coding gene *ews* in Ewing's sarcoma; *aml-1* is fused to the transcription repressor-coding gene *eto* (*mtg-8*) in several cases of acute myeloid leukemia. RARα is fused to the zinc finger/LZip-containing PML transcription factor in most cases of PML. The effect of this abnormal protein is to arrest myeloid differentiation in cells exposed to physiological levels of t-RA, the RARα ligand. Higher, pharmacological levels of t-RA overcome this block, which may reflect the fact that leukemia cells are heterozygous for the translocation. Normal receptors, encoded by the nontranslocated *RAR*α allele, are probably functionally repressed by the fusion gene so that higher levels of t-RA are required for activation. *Hrx/enl* and *Hrx/af-4* are hybrid transcription factor-coding genes (>30 examples of *Hrx*-containing fusions have been described today) found in some acute leukemias (41–43). The CBP coactivator HAT is required for transcriptional activation mediated by many GSTFs, including glucocorticoid receptor, RARs, c-Jun, c-Fos, and c-Myb, thus playing an important role in the control of cellular proliferation (27). A fusion gene consisting of CBP-coding sequences (including its HAT domain) from chromosomes 16p13 and 8p11 sequences encoding a putative coregulator designated monocytic-leukemia zinc finger protein has been associated with M4/M5 acute myeloid leukemia (44). In phenotypically similar acute myeloid leukemias with an inv(8)(p11;q13), monocytic-leukemia zinc finger protein is fused to the NR coactivator transcription intermediary factor 2, which itself interacts with CBP and is predicted to have intrinsic HAT activity (45). This suggests that gene rearrangements involving transcription coregulators may also contribute to the origin and/or progression of neoplastic diseases (indeed, this is one of the most important emerging themes in pathogenesis of hematological malignancies). Translocation-produced chimeric transcription factors are truly tumor specific and, as such, provide excellent targets for the design of small molecule drugs, which might hasten the development of highly selective and, therefore, less toxic forms of leukemia therapy (46). In addition, the remarkable, recently recognized connection between chimeric transcription factors in leukemia, particularly acute myeloid leukemias that display a prominent block in cellular differentiation, and alterations in regulation of histone acetylation may broaden the spectrum of rational pharmacological manipulations.

There are also some GSTFs that are potential oncogenes or affect directly the action of known proto-oncogenes.

NF-κB is a heterodimer of proteins that belong to the Rel family of GSTFs. Members of this family include the proto-oncogene products c-Rel, p50/p105 (NF-κB 1), p65 (Rel A), p52/p100 (NF-κB 2), and Rel B (47). It is noteworthy that the products of two other oncogenes, *lyt-10* and *bcl-3*, are also related to NF-κB and its inhibitory protein I-κB, respectively, implying that aberrations affecting multiple members of the Rel/NF-κB family can lead to cell transformation. NF-κB is a key regulatory molecule that is activated in response to a plethora of stimuli (*e.g.*, mitogens, cytokines, viral/bacterial products, and various types of stress; Ref. 47). Moreover, NF-κB seems to affect cell survival and determines the sensitivity of cancer cells to cytotoxic agents, as well as to ionizing radiation. Therefore, interfering with the protective role of NF-κB may result in chemo- and radio-sensitization of cancer cells (48). To this end, the resolved three-dimensional structure of the multifunctional Rel-homology domain of NF-κB (49) provides a matrix for selective targeting by small molecule drugs.

C-*erb*B-2 is a proto-oncogene encoding a single-chain receptor tyrosine kinase that is overexpressed in 25–30% of breast and other solid tumors and is associated with poor prognosis and a reduced response to conventional chemotherapy regimens. It has been shown that the AP-2 transcription factor is involved in c-*erb*B-2 up-regulation in human mammary carcinoma. In this regard, it has been demonstrated that the expression of c-*erb*B-2 can be inhibited effectively by molecules interfering with the DNA-binding activity of AP-2 (50, 51).

II. ER. ER is a member of the superfamily of NRs, which, in the absence of its ligand, resides in the cytoplasm complexed with heat-shock proteins (52). Once bound by estrogens, the receptor undergoes conformational changes, allowing dimerization, nuclear translocation, and high-affinity binding to specific response elements on target gene promoters. Estrogens induce breast cancer cell proliferation by up-regulating the expression of the genes for TGF-α and IGF-I. Tamoxifen, a widely used antiestrogen for breast cancer therapy, inactivates AF-2 of ER, while potentiating AF-1 (53). Consequently, tamoxifen inhibits the expression of TGF-α in breast cancer cells but stimulates IGF-I in endometrial cells and TGF-β3 in bone, thereby exerting beneficial effects in breast cancer and osteoporosis treatment, while representing a risk for cancer in the female reproductive system. However, another estrogen analogue, raloxifene, is capable of exerting its beneficial effect in breast cancer and preserving bone mass, without any endometrial side effects (54). Since 1995, it is known that there are two subtypes of ER, ERα and ERβ, exhibiting only small differences in their functional domains/regions. The discovery of ERβ might solve some long-standing puzzles concerning estrogen action in carcinogenesis, whereas it could have an impact on the diagnosis and treatment of breast cancer (*e.g.*, 5–10% of women with ER-negative metastatic breast cancer respond to tamoxifen therapy, a finding suggesting that these tumors are richer in ERβ; Ref. 55). Several pharmaceutical companies are aiming their research at the ER field, especially for the development of

ligands selective for ERα and ERβ to obviate the aforementioned side effects.

III. Tumor Suppressor Genes. In the year 2001, there were ≥24 tumor suppressor genes defined strictly by the observation that germ-line mutation of these genes predisposes to human cancer (56). Some of these genes encode GSTFs.

The *RB1*-gene product functions as transcriptional modulator by making a complex with the E2F family of GSTFs. E2F regulates the expression of a number of genes associated with cell proliferation and DNA synthesis, including the *myc* and *myb* proto-oncogenes and the genes encoding thymidine kinase, dihydrofolate reductase, and DNA polymerase. Binding to RB1 blocks the function of E2F as a transcriptional activator, and the RB1-E2F complex acts instead as a transcriptional repressor (57). E2F may not be the only target of RB1 or RB-related proteins. It seems, *e.g.*, that p107 (an RB-related protein) can bind also to the transactivation domain of Myc and suppress its activity (58).

WT1, the WT gene product, is a sequence-specific, DNA-binding protein that acts as a repressor of growth-promoting genes. Among the targets for repression is the gene encoding IGF-II, which is characteristically overexpressed in WT and is thought to act as an autocrine growth factor in the development of these neoplasms (59).

As the most intensely studied protein, p53 possesses myriad potential functions, whereas hundreds of mutations bring about losses and gains of functions further diversifying p53 behavior. This diversity, along with divergent signals that modulate p53, further increases the complexity of p53. The p53 tumor suppressor is a GSTF involved in cell cycle checkpoints, apoptosis, and genomic stability (60). p53, however, has activities that are independent from its transactivating function. Thus, p53 can transrepress viral and cellular promoters and can induce a transcription-independent apoptosis (61). It has now become clear that the activity of p53 is regulated primarily at the level of protein stability, although the switch from latent to active forms of the p53 molecule can also play an important role (62). Oncogenic mutations in p53 that abrogate its transactivating function lead to a stabilization of mutant p53 because of the disappearance of the p53-inducible proteins, which otherwise directly (Mdm-2) or indirectly (p21WAF1/Cip1) target p53 for degradation (61). Recently, two p53 relatives, p63 and p73, have been identified. Although both appear to contribute to normal growth, neither has shown yet strong correlation with tumor development (63). All members of the p53 family contain oligomerization domains and form homotetramers, which seems to be necessary for efficient transcriptional activity. However, the ability to oligomerize can also lead to inactivation of the wild-type protein when complexed with a mutated p53 form. Although not all p53 mutants behave this way, this activity underlies the gain-of-transforming function exhibited by some mutant p53s in the absence of wild-type protein. The solved three-dimensional structure of p53, alone or in complexes with interacting biomolecules, can assist greatly the discovery of novel small molecule compounds that fit selectively into functionally defined sites of the protein.

Retinoids play an important role in cell development, differentiation, and homeostasis. The discovery of retinoid receptors has revolutionized our understanding of the pleiotropic effects of these structurally simple molecules. A major source of diversity in the control of gene expression by retinoids originates from the existence of two subgroups of RARs, the RAR isotypes (α, β, and γ) and the retinoid X receptor isotypes (α, β, and γ). Transcriptional cross-talk and the finding that these receptors interact with multiple coregulators generate additional levels of complexity (17, 64). Retinoids are of particular pharmacological interest, as they show great promise for both prevention and treatment of various types of cancer (65, 66). Accumulating evidence suggests that abolishment or reduction of RARβ expression may result in loss of growth regulation and tumor development in various tissues (*e.g.*, breast and lung cancer, premalignant oral lesions, cervical, ovarian, and prostate cancer; Ref. 67). Thus, the *RAR*β gene could be considered as a potential tumor suppressor gene.

IV. Cell Cycle Regulators. The normal mammalian cell cycle comprises four temporally distinct phases. One important checkpoint is the restriction point in late G_1 phase (68). Passage through this phase is promoted by a group of G_1 cyclins, which include the mid-G_1, the D-type cyclins, and, in late G_1, cyclin E. These cyclins can heterodimerize with specific catalytic subunits, the CDKs, to form holoenzymes. Some substrates of these holoenzymes, which are inactivated on phosphorylation, are the RB1 protein and the RB-related proteins p107 and p130. The ability of the cyclin/CDK holoenzymes to phosphorylate RB1 is inhibited by a family of small proteins, known as CDK inhibitors. Increased abundance of cyclin/CDK complexes can directly affect gene expression by altering the activity of specific transcription factors and the function of the BTA (69).

Several GSTFs binding to G_1 cyclin gene promoters have been identified. The cyclin D1 promoter binds AP-1 proteins, E2F-p107, and Sp1-Sp3, whereas Ets proteins function to modulate proximal promoter activity (70, 71). Putative transcription factor-binding sites have been also identified in the cyclin D2 (AP-2, PUF, and Sp1) and cyclin D3 promoters (AP-2 and Sp1; Ref. 72).

Transcription Factor-targeted Drugs: The "Centripetal" Approach to Antioncogenesis

The knowledge resulting from understanding the mechanisms that govern gene expression can be used for designing drugs affecting tumor-specific transcriptional patterns. Much of the current anticancer research effort is focused on cell-surface receptors because they provide the easiest route for drugs to affect cellular behavior, whereas the agents acting at the level of transcription need to penetrate the cell membrane. However, the therapeutic effect of manipulations on surface receptors should be considered less specific, because their actions are mediated and modulated by complex signal transduction pathways that cross-talk frequently with signals controlled by other receptors. Given the "chaotic" nature of cancer, the design of a drug targeting a surface/cytoplasmic molecule has the disadvantage that a number of downstream protein network defects could occur during the carcinogenesis process, thus "neutralizing" the upstream therapeutic effect. This biological factor renders the development of such drugs a complicated and laborious procedure and should always be taken into account. On the other hand, tackling at the GSTF-DNA/GSTF coregulator interaction

level bypasses all of the intermediate steps from the site where "decisions are taken" (*i.e.*, interaction between surface receptor and corresponding ligand) to the site where "decisions are executed" (*i.e.*, binding of the GSTF to cognate DNA element and/or coregulators/mediators). The nature of GSTFs, their combinatorial interplay, and the heterogeneity of DNA-response elements in the respective target genes, as well as the number of structural regions that are amenable to regulation, provide the opportunity for selective intervention in a broad spectrum of neoplastic diseases. To this end, small molecule drugs directed against a functionally defined, GSTF site of interest can be developed, so that although still synthesized, the ability of the transcription factor to affect its target gene(s) is impaired drastically. A novel nucleic acid-based phenomenon, termed RNA interference, may be used, along with the increasing experience in antisense oligonucleotide applications (73), as a method to rapidly test whether a candidate GSTF is indeed a good target for the development of small molecule ligands interfering with its function in malignant cells (74). This method incorporates an initiation step where input double-stranded RNA is digested into 21–23-nt siRNAs and an effector step where the siRNA duplexes bind to a nuclease complex (RNA-induced silencing complex), which is responsible for digestion of the homologous target mRNA (75). In this context, by placing a gene fragment encoding the appropriate double-stranded RNA (*i.e.*, parts of the GSTF under investigation) under the control of an inducible promoter, one should be able to inactivate GSTF-regulated genes at particular cellular settings. To this end, gene-specific silencing by introducing siRNA duplexes into cultured mammalian cells has been reported recently (76).

In addition to improved selectivity, small molecule drugs, defined by a molecular mass < 500 kDa, have many potential advantages over larger molecular mass anticancer agents (*e.g.*, easier uptake into poorly vascularized regions of tumors and intracellular penetration; Ref. 77).

Modes of Action of Transcription Factor-targeted Small Molecule Drugs

The modular architecture of GSTFs makes them vulnerable to the action of small molecule drugs. A small molecule drug may exert its action by interacting specifically with the DNA-binding/dimerization domain, the transeffecting domain, or another domain/region that regulates a defined biochemical function (Fig. 2; Ref. 2). The mode of action of the drug could be simple steric hindrance of GSTF homo/heterodimerization, GSTF-DNA, or GSTF-other protein (*e.g.*, BTF, coregulator/mediator) interactions, resulting in transcriptional inhibition of specific genes (Fig. 2, *parts 2–5*). Alternatively, the drug might act "allosterically" (*i.e.*, preventing a crucial conformational change in a functional domain of the GSTF, resulting in an unstable interaction of the GSTF with its partners in a given transcriptional complex). Thus, gene expression could either be decreased (*e.g.*, inactivation of a transcriptional activator) or increased (*e.g.*, inactivation of a transcriptional repressor). Other possible actions of the drug might be inhibiting or mimicking (de)phosphorylation events critical for the function of GSTF domains/regions, affecting the charge/redox state of important amino acid residues, "masking" its nuclear localization sequence, or reducing its half-life by enhancing its degradation (Fig. 2, *parts 1* and *6*).

In addition to the above, translocation-generated hybrid transcription factors bear novel amino acid stretches, hence, unique local tertiary structure, at the junctions between the fusion partners, which are not found in normal cells. The configuration of these regions provide tumor-specific targets for the design of small molecule drugs. Such agents would complex with the hybrid transcription factor and inhibit, directly or allosterically, its novel DNA-binding/transeffector capacity or enhance its degradation, thus preventing aberrant expression of cognate genes. A decisive advantage of such an approach would be the reduced likelihood of the outgrowth of resistant cells, a major liability in contemporary forms of cancer chemotherapy. Recent reports using a tyrosine kinase-specific inhibitor to antagonize BCR-ABL function in chronic myeloid leukemia highlight the potential use of tumor-specific gene fusions as therapeutic targets (78). Altered interactions with transcriptional corepressors are a fundamental part of the transforming properties of chimeras, such as PML/RARα, offering complementary routes for pharmacological interventions. Although candidate target genes are beginning to be identified for many hybrid transcription factors, their immediate biochemical effects remain largely unknown. As new technologies facilitate the identification of candidate target genes (*i.e.*, DNA chip and array technologies), it will be critical to define which target genes and pathways specific chimeras alter and whether these are unique to each oncoprotein or whether there are common targets that could be manipulated pharmacologically. Similarly, the recent gain in understanding of coactivator-associated acetylation or deacetylation functions at the molecular level sets the ground for new strategies of pharmacological interference within transcriptional complexes exhibiting altered substrate specificity. There is one case report describing the outstanding clinical response of a t-RA-resistant patient with acute PML to t-RA combined with phenylbutyrate, a relatively weak HDAC inhibitor (79). It is anticipated that, during the next 10 years, there will be an enormous expansion of research in this area that should determine whether rational inhibition of HAT and/or HDAC activity is a viable therapeutic strategy in "reprogramming" patients with specific, genetically defined subtypes of leukemia.

Mutations of p53 provide both challenges and opportunities in cancer therapy. The most promising approach is a small molecule drug-induced restoration of normal functions of mutant p53. Not only may it not affect normal cells with wild-type p53, but also, it may be especially toxic for cancer cells with mutant p53 because of a sudden acquisition of function by stable and, therefore, overexpressed mutant p53. Once the function is restored, p53 will be degraded rapidly. Alternatively, instead of its restoration, loss of p53 function in cancer cells can be exploited for therapeutic advantages. Thus, small molecule drug-mediated inactivation of wild-type p53 in normal cells may be protective against radiation. Mdm-2-mimicking or Mdm-2-inducing small molecule drugs that enhance Mdm-2-dependent degradation of p53, as well as drugs impairing p53 tetramerization, may be developed for nontoxic regimens (61, 80).

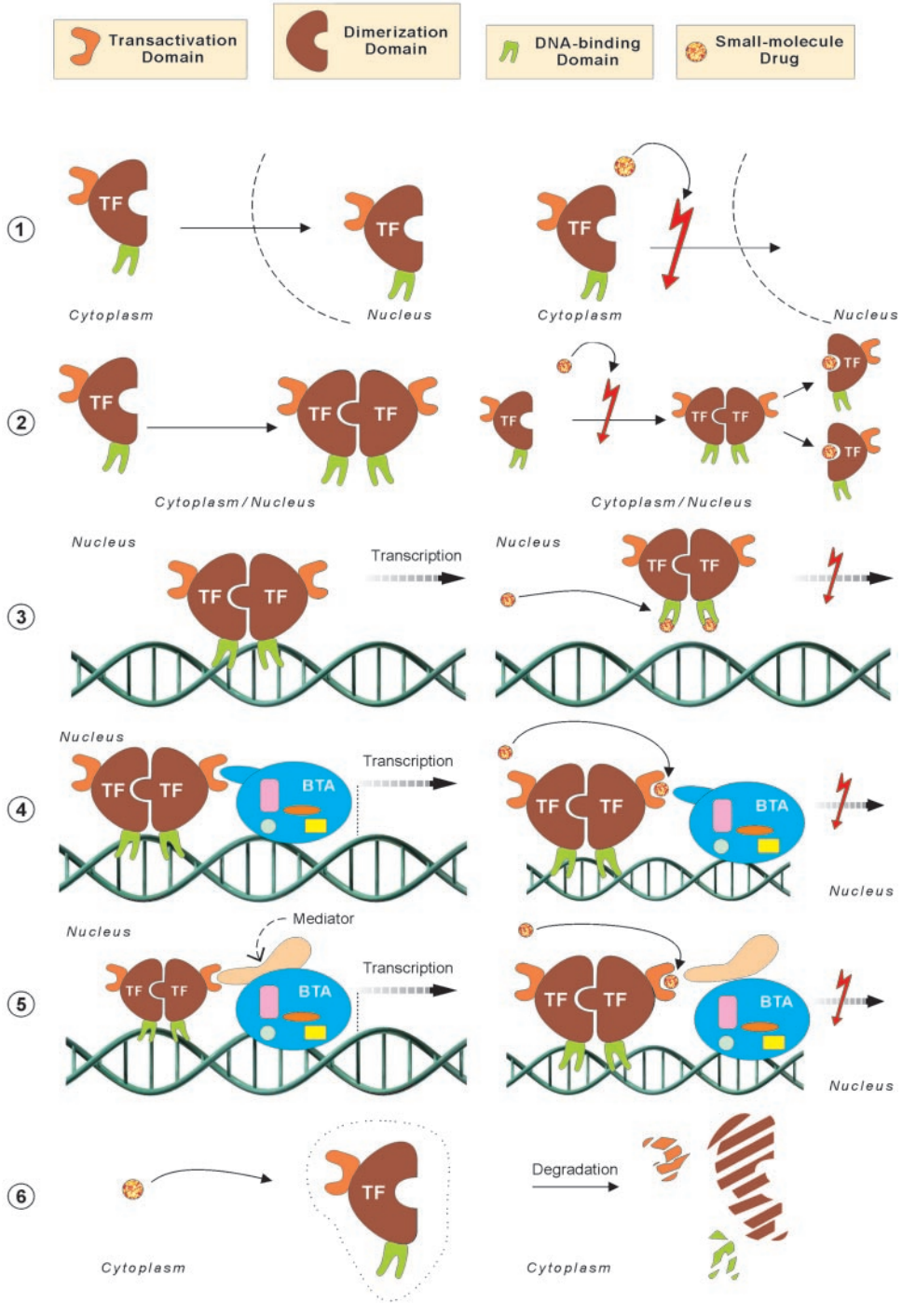

Fig. 2 Modes of action of GSTFs (*TF*) and possible ways in which rationally designed small molecule drugs could influence their function. An NR is taken as an example. In *1*, the putative drug may interfere with the nuclear translocation of the TF, precluding its physical contact with DNA/BTA, hence, overall activity. In *2*, most TFs need to form dimers/oligomers to be active. The putative drug may prevent the dimerization/oligomerization process, thereby impairing TF activity, although nuclear localization may be accomplished. In *3*, after dimerization, TFs bind to promoter/enhancer regions to modulate transcription of respective genes. Drugs designed to hinder DNA binding may result in inhibition of the transcription process. In *4*, other potential drugs may interfere selectively with the interplay of TFs with the BTA (pol II and BTFs), leaving DNA binding intact. In *5*, a variety of coregulators/mediators, with positive or negative action, has a pertinent role in the activity of several TFs. These "bridging" molecules seem to contribute critically to the DNA-binding and/or transeffecting potential of selective TFs in different cell types, leading to different transcriptional specificities and roles. Revealing the molecular identity of these mediators and their precise mode of action in the TF-DNA-BTA complex may provide unique TF biosurfaces for small molecule drug targeting. Finally, in *6*, TF degradation may also be subject to small molecule drug targeting. Enhancing oncogenic TF degradation could minimize its deleterious effects.

Developing Transcription Factor-targeted Small Molecule Drugs

I. Current Strategies. The above considerations have led several biotechnology and pharmaceutical companies to carry out large-scale drug screens, in an effort to discover small molecule agents that target various elements of the transcriptional machinery. The strategy for identifying these compounds has two branches.

The first corresponds to high-throughput screening procedures that will examine available industrial compound libraries. These pharmaceutical libraries are actually comprised of natural extracts mostly derived from plants, fungi, and marine organisms, because their synthesis by conventional organic chemistry methodologies is a tiresome task. They contain a wide spectrum of perplexing molecules with little prior knowledge of their putative biomolecular targets.

Table 1 Examples of antitumor agents that regulate/interfere with the transcriptional machinery[a]

Agent	Chemistry/origin	Mechanism of action[b]	Reference
Tamoxifen (antiestrogen)[c]	(Z)-2-[4-(1,2-Diphenyl-1 butenyl)phenoxy]-*N*,*N*-dimethylethanamine. Derived from soy	Interferes with ER transcriptional activity by competitively inhibiting estradiol binding and inducing conformational changes in the receptor, preventing its interaction with its coactivators	83
GW5638 (antiestrogen)[c]	3-[4-(1,2-Diphenylbut-1-enyl)phenyl]acrylic acid	Unique conformational change in the ER. Alternative therapy in tamoxifen-resistant breast tumors	84
ICI 182, 780 (antiestrogen)	7 α-alkylamide analogue of estradiol	Interaction with ER, through an unidentified allosteric binding site, that results in ER activation and subsequent ER down-regulation	85
W7	Naphthalenesulphonamide (calmodulin antagonist)	Blocks ER-estrogen response element interaction. Alternative therapy in tamoxifen-resistant breast tumors	86
Analog II (AII)	Z-1,1-dichloro-2,3-diphenylcyclopropane	Interaction with type II estrogen-binding sites and subsequent regulation of transcription	87
Retinoids[c]		Activation of retinoic acid response element-mediated transcription and inhibition of AP-1	88
Trichostatin A & leptomycin	Derived from *Streptomyces spp*	HDAC inhibitors	89
Oxamflatin	Aromatic sulfonamide hydroxamate	HDAC inhibitor	90
MS-275	Synthetic benzamine	HDAC inhibitor. Selectively induces the expression of TGF-β type II receptor, which is transcriptionally repressed in many human tumors	91
SAHA	Suberoylanidehydroxamic acid	HDAC inhibitor. Activates p21WAF1/Cip1 in a p53-independent manner, through the two Sp1-binding sites located in the promoter of the *WAF1* gene	92
FR901228	Bicyclic depsipeptide (fungal metabolite)	Transcriptional regulation of specific genes via deacetylation of histones	93
Aminothiol WR-1065	Aminothiol	Protects normal tissues from the toxic effects of certain cancer drugs while leaving their antitumor actions unaltered. This effect is mediated through enhanced binding of NF-κB, AP-1, and p53 to their cognate DNA elements	94
Parthenolide	Sesquiterpene lactone parthenolide from the anti-inflammatory medicinal herb Feverfew (*Tanacetum parthenium*)	Mimicks the function of I-κBα, inhibits I-κB kinase, and blocks STAT3 (signal transducer and activator of transcription 3) activation	95–97
Daunomycin	Anthracycline	Induction of p53, partially through binding of NF-κB to the NF-κB response element of the p53 promoter	98
Paclitaxel[c]	Taxane derived from the plant *Taxus Brevifolia*	Degradation of I-κB and subsequent activation of NF-κB	99
Ajoene	Compound of garlic	Activates NF-κB	100
Sulphur mustards		Inhibit the DNA-binding activity of transcription factor AP-2	101
Distamycins	Polyamide	Inhibit the DNA-binding activity of transcription factors OTF-1 and NF-1	102
Ecteinascidin-743 (ET-743)	Marine tetrahydroisoquinoline alkaloid isolated from *Ecteinascidia turbinata*	Promoter-specific blocking agent interfering with the DNA-binding activity of various transcription factors, including E2F, serum response factor, and NF-Y	103, 104
Staurosporine	Derived from *Streptomyces staurosporeus*	Inhibits I-κBα and I-κBβ, subsequently leading to activation of NF-κB	105
Rituximab[c]	Anti-CD20 monoclonal antibody	Decreases the ability of transcription factor STAT3 to bind to its cognate DNA element	106
Piceatannol	Phytochemical stilbene from *Euphorbia lagascae*	Inhibition of transcription factors STAT3 and STAT5	107
Rapamycin & analoges (CCI-779)	Macrolide	Inhibits the protein kinase mammalian target of rapamycin, thus blocking the activity of the ribosomal S6 kinase and the function of the eukaryotic initiation factor 4E-binding protein-1. In addition, it prevents CDK activation and inhibits phosphorylation of RB, thus blocking the activity of E2F-1	108
E7070	Sulfonamide	Suppresses the expression of cyclins A and B1, CDK2, and CDC2 and induces the expression of p21WAF1/Cip1 and p53. In addition, it prevents the phosphorylation of RB, thus blocking activation of E2F-1	109
Mofarotene (RO40-8757)	Retinoid	Down-regulates, in a tumor-specific manner, the transcription of a mitochondrial gene encoding a subunit of NADH dehydrogenase.[b] In addition, it up-regulates CDK inhibitors p21WAF11/Cip1 and p27 and shifts RB in its hypophosphorylated form, thus blocking activation of E2F-1	110,111

[a] Inhibitors of the BTA, such as actinomycin D and rifamycins, are not included.

[b] In certain cases, the mechanism may be cell or tumor specific. Furthermore, whether the effect on transcription is direct via a GSTF or indirect, remains to be determined.

[c] Used in Phase III clinical trials.

A classical example from this process is the anticancer drug taxol.

The second branch is based on combinatorial chemistry and chemical genomics. In the former case, the process starts with a chemical compound called "platform" that binds onto a specific molecular target (*i.e.,* GSTF) and then continues with increment changes of the platform, generating many antitarget compounds and readily learning which of them is most appropriate in disrupting a particular biological pathway. In the latter case, low-resolution genomic technologies (*i.e.,* microarray technology) can provide genomic targets of interest. Then, the corresponding protein (tumor-related GSTF) can be screened with affinity selection methods to identify small molecule ligands (81, 82).

Representative examples of antitumor agents developed by exploiting various aspects of these strategies and that target certain components of the transcriptional machinery are given in Table 1.

II. Future Perspectives. *In silico* approaches, such as structure-based ligand design methodologies, hold great promise in the synthesis of small molecule drugs that alter the activity of cancer-associated GSTFs. Two factors underly the increasing importance and effort of structure-based drug design: (*a*) on the one hand, new computer programs/algorithms for conformational analysis, ligand docking, structural alignment, and *de novo* design have been developed (6, 112–114); and (*b*) on the other hand, enormous progress in the molecular biology providing the three-dimensional structure and structure-activity relationships of therapeutically relevant biopolymers. As an ever-growing number of solved GSTF three-dimensional structures are being added to the data pool, one future challenge of structure-based drug design will undoubtedly be to apply the arsenal of available tools to the design of GSTF-targeted, low molecular mass agents that would modulate specific functions with high selectivity (115, 116). Structure-based technologies have the advantage of looking at each transcription factor as an entire site of interest and considering several interactions with defined biomolecules, with different steric and electrostatic complementarities. At present, we are beginning to understand some important underlying principles and phenomena determining molecular recognition in protein-protein and DNA-protein interfaces, including plasticity of interacting partners, DNA-induced folding, protein-induced DNA distortion, such as bending or twisting, and cooperativity through interactions of DNA and protein domains. These parameters have to be incorporated efficiently into methods for lead structure discovery and ligand docking. Computational chemistry techniques of molecular modeling provide powerful tools to support the finding and optimization of new lead structures in terms of potency, selectivity, and drug-like properties (6, 117).

It can be expected that with the growing experimental data on structures (obtained by X-ray crystallography and multidimensional nuclear magnetic resonance spectroscopy) and thermodynamics of DNA/protein-protein complexes, the range of applicability of these strategies will be extended further. When an experimental GSTF structure is not available, homology modeling can be used to build a tentative three-dimensional structure based on a known protein structure of a GSTF that belongs to the same family, thus sharing a specific DNA-binding/dimerization motif.

III. Testing the Efficacy of Newly Developed Compounds. Ligands identified through any of the aforementioned strategies may be used in *in vitro* and *in vivo* assays to assess their biological activity toward the desired effect.

The *in vitro* assays include electrophoretic mobility-shift assays and reconstituted transcription reactions. These methods examine the ability of the putative anti-GSTF drugs to interfere with the function of DNA-binding/dimerization motifs or transeffecting domains. The major advantage of these assays is that they are highly reproducible and precisely controlled. Furthermore, their capacity can be scaled up to carry out large series of parallel screens that are required for the screening strategy described. However, *in vitro* screens represent the first step toward drug identification, as the specific actions of a lead compound in *in vitro* systems may fail to take place within intact cells. Because of these potential pitfalls, cell-based screening methodologies have been developed. The basic principle of these *in vivo* approaches is to monitor the transcriptional levels of reporter genes from a defined promoter, in the presence and absence of the putative compound. Traditionally, most of the reporter genes encode either enzyme activities that can be detected by colorimetric assays (*i.e.,* secreted alkaline phosphatase) or enzymes capable of catalyzing light-emitting reactions (*i.e.,* luciferase). A technical progress introduced recently in the screening strategy field promises a more sensitive and quicker method for recognizing promoter-specific inhibitors. This improvement is based on the use of "collision" constructs in the reporter assays (118). These constructs contain a reporter gene and two opposing promoters, which, in the absence of a specific inhibitor, dictate transcription toward each other and collide somewhere within the reporter gene, disrupting transcript production, whereas in the presence of the inhibitor, a positive substrate reaction is obtained. The main advantages of this assay are the following: (*a*) the inhibitory effect is converted into a positive detection signal; and (*b*) each drug test has a built-in positive control, designed to avoid false-negative interpretation because of the toxic effect of the compound on general components (*i.e.,* BTFs) of the transcriptional machinery.

Conclusions

Remarkable progress in the area of gene control mechanisms has begun to unravel the transcriptional circuitries that operate in malignant cells. These research advances offer insights into the types of transcriptional regulatory molecules that might be targeted by selective agonists or antagonists to correct abnormal gene function. Furthermore, precise mapping of the immensely complex signaling networks culminating in oncogenic transcription factors will provide indications of novel aspects of transcription, which might also be tackled (119). Innovative, GSTF structure-based small molecule drugs with increased selectivity, thus, minimal side effects, will be created in the near future, by pinpointing the nuclear players that orchestrate cancer formation, maintenance, and progression. High-resolution functional genomics (*i.e.,* characterization of gene/protein function on a genome-wide scale) and proteomics (*i.e.,* the parallel separation and large-scale identification of novel

proteins) hold key positions in this endeavor by charting interaction partners of relevant GSTFs. Finally, pharmacokinetics problems (a major rate-limiting step in drug development) are being addressed pragmatically by high-throughput cassette-dosing approaches, thus allowing clinical trials to test mechanistic hypotheses, as well as fulfill the usual requirements.

Inasmuch as this approach attacks the "integration" center of an otherwise many faceted intracellular informatics problem, it may prove ultimately more effective in treating a large proportion of clinical cancers and their complications (120).

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