

Transcription Factors as Potential Targets for Therapeutic Drugs

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Abstract: Although drugs which target transcription are in wide therapeutic use, they were all identified on the basis of their effect on a specific biological process such as inflammation or hormone responses and were only subsequently shown to target transcription. Our recent progress in understanding the mechanism of action of these drugs and the mechanisms of transcriptional regulation in general offers hope for a new generation of drugs isolated on the basis of their ability to modulate either the synthesis of transcription factors, the regulation of their activity by ligands or phosphorylation events, their protein-protein interactions or their binding to DNA.

INTRODUCTION

Regulation of gene expression is central to the normal development and proper functioning of all organisms since it results, for example, in different proteins being made by different cell types allowing these cells to perform different functions (for reviews see 1,2). Such gene regulation is primarily achieved at the level of gene transcription whereby the DNA is copied into an RNA transcript. Although some regulation after transcription does occur (for review see 1), in general once transcription occurs the other stages of gene expression such as RNA splicing or translation into protein follow more or less automatically. Thus, transcription of different genes in different cell types leads to the production of their corresponding proteins whilst a particular stimulus such as cyclic AMP or steroid hormones will produce new protein synthesis by activating the transcription of genes which were not previously transcribed.

In view of this central role for transcription in biological processes, it represents an obvious target for therapeutic drugs which could act either by stimulating the transcription of specific genes required for a desired beneficial effect or by inhibiting the transcription of genes involved in an undesirable event. Indeed of the 50 FDA-approved best selling drugs, more than 10% target transcription and these include such well known drugs as salicylate and tamoxifen [3]. Interestingly however, these drugs were isolated in screens

designed to produce specific biological effects such as immunosuppression or inhibition of hormone action rather than by screens for drugs which directly target transcription. Subsequently, when the mechanism of action of these drugs was investigated, they were found to affect transcription.

The existence of such drugs indicates that transcription does represent a suitable target for therapeutic drugs. Moreover, our increased understanding of the mechanisms of action of these drugs can be used to offer insights into the types of transcriptional regulatory processes which might be targeted by new drugs. Similarly, our increased understanding of gene transcription in general provides indications of novel aspects of transcription which might also be targeted.

It is the purpose of this review to consider the manner in which such increased understanding of transcription in general and of the actions of transcription modulating drugs in particular could be used directly to identify new potential therapeutic agents modulating transcription on the basis of their ability to do this. This will provide a new level of cellular control processes which can be directly targeted for therapeutic benefit.

Regulation of Promoter Activity

Transcription of a specific gene is dependent upon an array of regulatory sequences known as the gene promoter which determines both the basal transcription of the gene and its response to specific stimuli. It is relatively simple to link this promoter to a reporter gene encoding a protein

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whose expression can be measured. The construct can then be introduced into cells and the effect of various agents on the activity of the promoter measured simply by measuring the expression of the reporter gene (by assaying the protein it encodes) in untreated cells and in cells treated with the different agents. This can be done in mammalian cells using, for example, the gene encoding the luciferase protein as a reporter, allowing a high throughput automated screen with luciferase activity being read in a luminometer [3]. This allows a wide range of compounds to be screened for their ability to stimulate or inhibit the activity of a particular promoter. Evidently, appropriate controls with a distinct promoter driving luciferase can be included to confirm that any effects observed are not due to a particular compound modulating luciferase mRNA stability, translation, etc. Such screening systems can evidently be used for identifying compounds regulating viral as well as cellular promoters allowing screening for anti-viral compounds [4]. Similar screens can also be set up in yeast using either a selectable marker or β -galactosidase to produce blue colonies when the promoter is active [5]. Obviously however, any drug identified in this way will need to also be shown to produce a similar effect in mammalian cells.

Although this approach has the advantage of high throughput, it suffers from the disadvantage that it will not indicate in any way the manner in which the particular compound which is identified modulates promoter activity. Hence, further studies will be required to determine the mechanism of action of the compound, both to indicate its potential side effects and to allow rational design of more effective derivatives. Moreover, this approach does not take advantage of any knowledge which is available as to the processes which regulate the gene of interest.

Transcription Factors: Synthesis and Activation

Transcription is controlled by regulatory proteins known as transcription factors which bind to specific DNA sequences in the gene promoter and activate or inhibit transcription (for review see 2). The critical importance of such factors is indicated by the fact that mutations in the genes encoding them result in a wide range of human diseases ranging from developmental disorders such as aniridia or Rubinstein-Taybi syndrome to cancers such as leukaemia or retinoblastoma (for review see [6]. Most importantly, transcription

factors play a key role in regulating the expression of specific genes in specific cell types or in response to specific stimuli. This is achieved by regulating the transcription factor itself so that it is either synthesized only in one particular situation or alternatively is activated from a pre-existing inactive form by some post-translational modification. Hence, the factor can only regulate the genes when it is present in a particular cell and is in an active form [2].

Clearly, either the synthesis of a transcription factor or its activity could be targeted for therapeutic benefit. In terms of synthesis, the same techniques could be used as for any other protein whose synthesis needs to be modulated. Thus, for example, considerable progress has been made in the development of modified anti-sense oligonucleotides, which are complementary to the mRNA encoding a specific protein, and which can be used therapeutically to inhibit its synthesis [7]. Although most such anti-sense studies have been conducted in cultured cells, this approach has been applied to target the synthesis of the transcription factor NF B in an *in vivo* animal model of experimental colitis and was shown to be a more effective means of treatment than treatment with glucocorticoid hormone which is normally used to interfere with NF B activity. [8]. Similarly, gene delivery can be used to deliver the gene encoding a transcription factor in the same manner as with any other gene in a gene therapy procedure and this method has been used, for example, to deliver the gene encoding the anti-oncogenic transcription factor p53 in a successful attempt to inhibit tumour growth [9]. Obviously, as in all such gene therapy approaches, the effectiveness of this approach will depend on the development of safe and efficient gene delivery systems for human use. Hence, transcription factor expression can be manipulated pharmacologically or by gene therapy in exactly the same manner as any other protein.

Evidently, however, such methods simply mimic the methods used to control the synthesis of any protein rather than taking advantage of the unique properties of transcription factors. This approach is likely to be of use therefore only in a situation where it is desirable to switch on or off an entire bank of genes involved for example, in inflammation, which are modulated by a particular transcription factor. In such cases, it would evidently be more effective to target the synthesis of the factor itself rather than each of the proteins encoded by its target genes. In many situations however, it will be preferable to develop ways of modulating the activity of a transcription factor.

Indeed, all the currently used therapeutic drugs target this aspect and the processes which they target or which could be targeted will now be discussed.

Ligand Binding

A number of transcription factors involved in activating or repressing genes in response to a specific signalling molecule are themselves activated from a pre-existing inactive form by direct binding of the ligand. A classical example are members of the nuclear receptor family. Individual members of this family are activated by binding of, for example, glucocorticoid, oestrogen or thyroid hormone and they undergo a conformational change which allows them to activate their target genes [10].

The important role of one of these hormones, oestrogen, in the growth of breast cancer cells has led to particular attention being given to the development of anti-oestrogens which could inhibit its activity by, for example, binding to the receptor, thereby preventing the binding of oestrogen, but not activating the receptor. One of these inhibitors, tamoxifen, which is in clinical use in breast cancer as an anti-oestrogen can also however, have an undesirable oestrogen-like activity in some situations [11,12].

Clearly, the improved understanding of the structure of the ligand binding domains of the nuclear receptors [13], both prior to and after binding of hormone and the manner in which this affects their interactions with co-activators or co-repressors, which is currently being obtained will facilitate the design of drugs which bind to the receptor but do not mimic any of the effects of oestrogen so having a pure anti-oestrogenic effect. Similarly, the recognition that one consequence of the conformational changes which occurs upon oestrogen binding to its receptor, is the binding to the receptor of co-activator proteins, which are necessary for transcriptional activation [14,15], indicates that another approach would be to develop drugs which block the protein-protein interaction between the receptor and its co-activator(s) (see below).

Phosphorylation

Although transcription factors can be regulated directly by ligands such as oestrogen which can enter the cell, many other signalling molecules

which cannot do so, set off cascades of kinase and/or phosphatase enzymes which ultimately results in the phosphorylation or dephosphorylation of one or more transcription factors resulting in their activation [16]. Several clinically-used drugs target this aspect by modulating the phosphorylation state of one or more transcription factors. Thus, cyclosporin and FK506 (tacrolimus) have an anti-inflammatory effect because they prevent the enzyme calcineurin from dephosphorylating the NF-AT transcription factor, such dephosphorylation being required for it to stimulate the expression of several genes involved in the immune response [17,18,19]. Similarly, salicylate inhibits the activation of the NF- κ B transcription factor by preventing the phosphorylation of the I κ B transcription factor [20] which is associated with the NF- κ B factor and inhibits its activity. Such phosphorylation is required for the release of I κ B from NF- κ B and prevents it activating its target genes which are involved in immune and inflammatory events [21].

Obviously, these drugs were identified on the basis of their ability to modulate biological processes such as the immune response rather than their effect on transcription factor phosphorylation. Therefore, our improved understanding of the effects of transcription factor phosphorylation and the enzymes which regulate it should allow the setting up of high throughput *in vitro* screens aimed at identifying compounds which modulate the phosphorylation/dephosphorylation of a particular factor by a particular enzyme. Such screens will be particularly valuable if they can identify compounds which can specifically modulate the phosphorylation of a target transcription factor by a specific enzyme without affecting the ability of the enzyme to phosphorylate other transcription factors, which may produce undesirable side effects. Thus, although NF-AT is expressed only in T cells, FK 506 and cyclosporin have toxic side effects due to their effects on other tissues which are presumably due to their effects on other transcription factors [22-24].

Protein-Protein Interactions

Interactions of transcription factors with other proteins are central to the regulation of their activity and the mechanism of their action. Thus, the NF- κ B factor discussed above, is an example of a factor whose activity is regulated via interaction with the inhibiting I κ B factor [21] whilst the nuclear receptors need to interact with co-activator

proteins to activate transcription [14]. Similarly, in order to ultimately stimulate transcription, activating factors or their co-activators need to interact with the proteins of the basal transcriptional complex to stimulate its activity [25].

Such protein-protein interactions represent an obvious target for disruption for therapeutic purposes. Thus, once the site of interaction on one or other of the interacting proteins has been mapped, a short peptide prepared from this region can be prepared *in vitro* and then in intact cells. For example, by using a peptide which disrupted the interaction between the cellular transcription factor Oct-1 and the herpes simplex virus (HSV) transactivator protein VP16, it was possible to inhibit the HSV lytic cycle in intact cells [26]. Similarly, heterodimerization between the E2F and DP-1 transcription factors could be inhibited using a peptide prepared from the interacting region resulting in a failure of DNA binding which is dependent upon heterodimerization, leading to apoptosis of tumour cells treated in this manner [27]. Hence, peptides can be used to target protein-protein interactions between transcription factors.

Although such peptides are themselves unlikely to be of use as therapeutic agents *in vivo*, their structure can serve as a basis to design therapeutically useful peptide mimetics which could be delivered *in vivo* (for review see 28). Thus, this approach has been used, for example, in the design of non-peptide inhibitors of the human immunodeficiency protease based on structural analysis of peptide inhibitors which bind to its substrate-binding site. Hence, this area holds considerable promise for the future.

DNA Binding

Obviously, binding to a specific binding site in DNA is necessary for the action of many activating or inhibitory transcription factors, and thus represents an obvious target for therapeutic drugs. Indeed, as noted above, the inhibition of E2F/DP-1 heterodimerization actually achieved its effect by preventing DNA-binding of the factor which required heterodimer formation. However, it is also possible to think in terms of drugs which interact with the binding site of a transcription factor in the DNA to prevent its binding. However, although a number of DNA-binding drugs such as distamycin have been developed to inhibit DNA replication as a means of cancer therapy, in

general, these do not have sufficient sequence specificity to specifically target the DNA binding site of a particular transcription factor. However, numerous attempts are being made to produce derivatives with greater specificity [29,30] utilizing improved structural information on the binding of these drugs to DNA [31,32].

An alternative approach, however, comes from the observation that specific sequences in double-stranded DNA can be bound by a single-stranded oligonucleotide to form a triple helix structure which is not recognized by a protein such as a transcription factor that would normally bind to that site in double helical DNA. This approach has been used for example, to inhibit the transcription of the *c-fos* proto-oncogene [33] or that of the tumour necrosis factor gene [34] which in the latter case resulted in inhibition of the growth of TNF-dependent tumour cells [34].

This approach, may thus, offer an effective means of specifically inhibiting gene expression and may in future be combined with the use of DNA-binding drugs particularly, since one such drug, distamycin, has been shown to differentially affect the stability of double helical versus triple helical DNA [35].

CONCLUSIONS

Many millions of people daily take therapeutic drugs such as tamoxifen, salicylate or FK506, which target transcription. Yet, none of these drugs was identified on the basis of this ability. Our increasing knowledge of the manner in which these drugs act at the level of ligand binding or phosphorylation as well as our improved understanding of protein-protein interactions and DNA binding by transcription factors, offers hope of a new generation of drugs isolated specifically on the basis of the ability to modulate transcription. These will arise both from high throughput screens of compounds for their effect, for example, on promoter activity or kinase activity and from designer approaches based on a detailed structural understanding of an individual transcription factor and its interaction with other proteins and with DNA.

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