tions. Assuming that the duration of action of serotonin at all of these receptors is regulated by reuptake, Prozac would be expected to affect them all. How does that lead to a reduction in the despondence of the depressed, alleviate anxiety in the fearful, and change the outlook of those who are sensitive to rejection? Perhaps selectivity comes because Prozac, by blocking reuptake, only augments the action of serotonin at those brain synapses where it is already being released. Does it, in this way, selectively strengthen already ongoing restorative mechanisms?

The problem is even more complicated, because the therapeutic effect of Prozac depends on adaptive changes in the brain that apparently take weeks to develop. This is suggested by the lag of up to a month before Prozac, imipramine, or many other chemically distinct antidepressants (including those that are selective norepinephrine reuptake inhibitors) become effective. Presumably their primary actions in prolonging neurotransmitter effects set into motion a series of molecular changes in the brain that may mitigate depression, alleviate anxiety, or alter temperament. But are these different psychological phenomena all alternative manifestations of the same underlying problem? Or are different adaptive changes put in motion in different underlying disorders? Explaining this chain of events is the most challenging current problem in psychopharmacology.

There are pressing clinical problems as well. Are the personality changes reported by Kramer and other clinicians really due to Prozac's pharmacological effects, or is the drug just an expensive placebo? Are the effects attributable solely to the drug or rather to its combination with some form of psychotherapy? Are the changes lasting? Must the drug be taken forever? Controlled clinical trials are needed, but both the critical therapeutic variables and the behavioral changes may be subtle and difficult to measure. And, since pharmaceutical companies are often reluctant to test such secondary applications, financial support for work of this type may be difficult to obtain. Yet there is a critical need to formally evaluate what are for now only persuasive, but unverified, clinical impressions about Prozac's efficacy.

But most important is the impact of these developments on the overall field of psychiatry. When chlorpromazine and imipramine were first introduced, they were initially popular only with a small subgroup of psychiatrists who called themselves biological psychiatrists and who tended to focus on serious mental illness, leaving other more common and less severe problems to those who specialized in psychotherapy. Now it is becoming generally appreciated that modern psychopharmacology, genetics, and other offshoots of biology are also relevant to an understanding of the less serious behavioral disorders. The fact that new enthusiasts for this position include Kramer and many others who had viewed themselves as being primarily psychotherapists signals a shift in the intellectual mainstream of this field. Whether Prozac ultimately proves to be of value in altering rejection sensitivity or low self-esteem, the new openness to biological treatment will have profound effects on the way we educate the next generation of psychiatrists and on our ability to attract the interest of biological scientists in psychiatric problems. In thinking about Prozac, we have been led to reevaluate our basic assumptions about behavioral disorders and how we approach them.

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Duality of TBP, the Universal Transcription Factor

Kevin Struhl

Transcription in eukaryotic organisms is extraordinarily complex. Three nuclear RNA polymerases are responsible for the synthesis of ribosomal (Pol I), messenger (Pol II), transfer (Pol III), and small nuclear (Pol II and Pol III) RNAs. These RNA polymerases act as structurally distinct promoters, and they function as part of macromolecular complexes composed of distinct sets of basic transcription factors. The Pol II machinery responds to numerous activator and repressor proteins, whose regulated action largely accounts for the diversity in gene expression patterns. Remarkably, there is a universal transcription factor, the TATA-binding protein (TBP), that is central to the expression of all eukaryotic genes. However, it appears that TBP does not play a common role in all transcription but rather has an inherent duality.

TBP is the most highly conserved eukaryotic transcription factor, with its functional domain showing greater than 80% sequence identity in a wide variety of species (*1*). It interacts specifically with TATA DNA sequences and with many proteins and carries out an impressive array of functions. First, TBP interacts with associated factors (TAFs) to form distinct multiprotein complexes, SL1 (*2*), TFIID (*3*), and TFIIIB (*4*), that, respectively, are specific for transcription by Pol I, Pol II, and Pol III. The relative ability of TBP to form these complexes is likely to regulate the balance of the various classes of RNAs in vivo (*5*). Second, for most Pol II promoters, specific binding of TBP to the TATA element initiates the assembly of an active transcription complex (*6*, *7*). In the course of this assembly process, promoter-bound TBP interacts with TFIIA and TFIIB, which are basic components of the Pol II transcription machinery. Third, TBP can interact in vitro with transcriptional activators (*8*) and general negative regulators (*9*), and it is likely to be a mechanistically relevant target of these and other transcriptional regulatory proteins in vivo. Fourth, TBP is a subunit of the SNAPc complex, which binds specifically to the proximal sequence element (PSE) of small nuclear RNA Pol II and Pol III promoters (*10*). Amazingly, all of these TBP functions are carried out by a single structural domain of only 180 amino acid residues.

As revealed by x-ray crystallography, TBP is an intramolecular dimer of related, but not identical, 90-residue subdomains (*11*). It has been described as a saddle consisting of a curved 10-stranded, antiparallel β sheet, with four α helices lying on its upper surface. Structural, biochemical, and mutational analyses indicate that the concave underside of the saddle binds to DNA, whereas the α helices and the convex surface of the saddle are likely to bind to other

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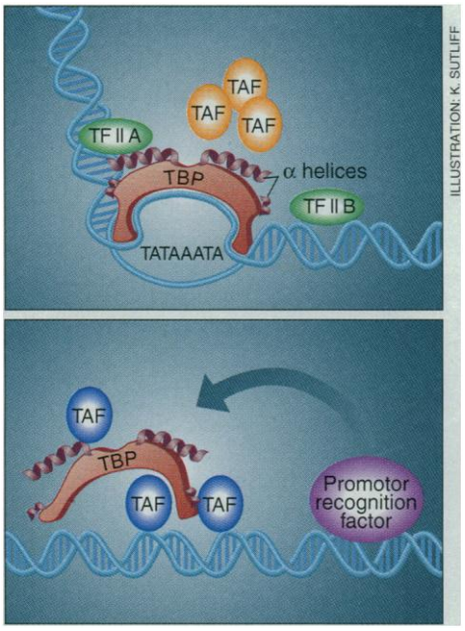
proteins. Although TBP and the TATA element have approximate twofold symmetry, the TBP-TATA complex has a preferred orientation that likely explains the unidirectionality of transcription (*12*).

The cocrystal structure of the TBP-TATA complex reveals a dramatic and unprecedented distortion in the DNA helix that is confined to the eight base pairs of the TATA recognition sequence (*12*) (see figure). Binding causes sharp (90°) kinks at the end of the TATA sequence, severe unwinding and compensating superhelical twist, and strong bending of DNA toward the major groove. The incoming and outgoing double helices are sharply angled (100°) and markedly displaced (by 18 Å), with the shallow and very wide minor groove of the TATA element interacting with most of the entire undersurface of the TBP saddle.

TBP displays unusual DNA-binding specificity in that it recognizes a variety of sequences that do not conform to a simple consensus (*13*). Thus, the ability of a particular sequence to undergo the requisite structural distortion may contribute more to DNA-binding specificity than functional complementarity between amino acids and base pairs. In this regard, minor grooves of DNA have comparatively few functional groups, and the TpA dinucleotide characteristic of TATA elements is relatively unstable and easily deformable (*14*). TBP and the Pol II–specific complex TFIID have nearly identical DNA sequence specificities (*15*) and hence are likely to form similar structures on the TATA element.

It seems inevitable that the unique structure of the TBP-TATA complex is important for the mechanism of transcription from Pol II promoters that contain TATA elements. The remarkable distortion at the TATA element could be propagated downstream, causing further unwinding and perhaps strand separation around the transcriptional initiation site. However, TBP is unlikely to directly propagate such effects because the distortion is topologically neutral and is limited to the TATA element, which is relatively far (generally 25 to 30 base pairs and even further in yeast) from the initiation site. Alternatively, the sharp DNA bend might bring other basic Pol II factors into closer proximity or promote the correct stereochemistry, possibly by facilitating the interaction of TBP with TFIIA or TFIIB. Finally, TBP bends the TATA element in a direction opposite to that preferred in nucleosomal DNA (*16*), suggesting a simple mechanism for the observed competition between histones and TBP, an important rate-determining step in transcription (*17*). TBP binding to nucleosomal DNA almost certainly would alter the local chromatin structure around the TATA element, which might facilitate access of other basic Pol II transcription factors or regulatory proteins.



Dual action of TBP. TBP participates in all eukaryotic transcription, but in some genes it dramatically distorts DNA structure (**top**), and in others it interacts indirectly with DNA (**bottom**).

Is the ability of TBP to bind and distort DNA relevant for transcription by Pol I and Pol III? Perhaps not. Pol I promoters and the vast majority of Pol III promoters lack TATA elements, and a TBP mutant that is defective for binding to TATA elements nevertheless supports Pol I and Pol III transcription in vitro (*18*). The TBP complexes SL1 and TFIIIB bind very poorly to DNA, and they are recruited to promoters via interactions with proteins (for example, UBF, TFIIIA, and TFIIIC) that are bound to Pol I– and Pol III–specific promoter sequences (*19*). Upon recruitment, both TFIIIB and SL1 contact DNA just upstream of the initiation site. However, these interactions with DNA are not sequence-specific and, in yeast TFIIIB, DNA appears to contact the TAFs, not TBP (*4*). Similarly, DNA contacts to the SNAPc complex involve the TAFs, and removal of TBP from the SNAPc complex does not prevent sequence-specific binding (*10*). Thus, TBP does not seem to interact with DNA when it is in the SL1, TFIIIB, and SNAPc complexes.

The inability of SL1, TFIIIB, and SNAPc to bind to TATA sequences indicates that, in these cases, the undersurface of the TBP saddle must be conformationally modified or occluded by the TAFs (or both) (see figure). However, an extensive surface of TBP required for Pol III transcription is clearly distinct from the DNA-binding surface, because it maps to the α helices and the convex side of the saddle (*5*). Hence, it is unlikely that TBP adopts a grossly different structure in the context of yeast TFIIIB. Thus, when TBP is coated with Pol I–, Pol III–, and SNAPc-specific TAFs, the concave surface of the saddle may interact loosely and nonspecifically with DNA.

From a structural and evolutionary perspective, it is appealing to imagine that transcription of all eukaryotic genes involves an interaction between TBP and promoter DNA. However, in actuality TBP likely has an inherent duality: The TBP-TATA complex mediates transcription from Pol II promoters containing TATA elements. (It may also be important at TATA-less Pol II promoters and those rare Pol III promoters that contain TATA elements.) In contrast, the TFIIIB, SL1, and SNAPc complexes utilize TBP in a form that either does not interact directly with DNA or interacts in a different manner. The combination of high-resolution structures of larger TBP complexes (that is, containing TAFs and basic transcription factors) and mutant TBPs with specific functional defects will be necessary to understand how TBP carries out its various transcriptional roles.

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