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Mechanism of DNA-binding enhancement by the human T-cell leukaemia virus transactivator Tax

[LETTER TO NATURE]

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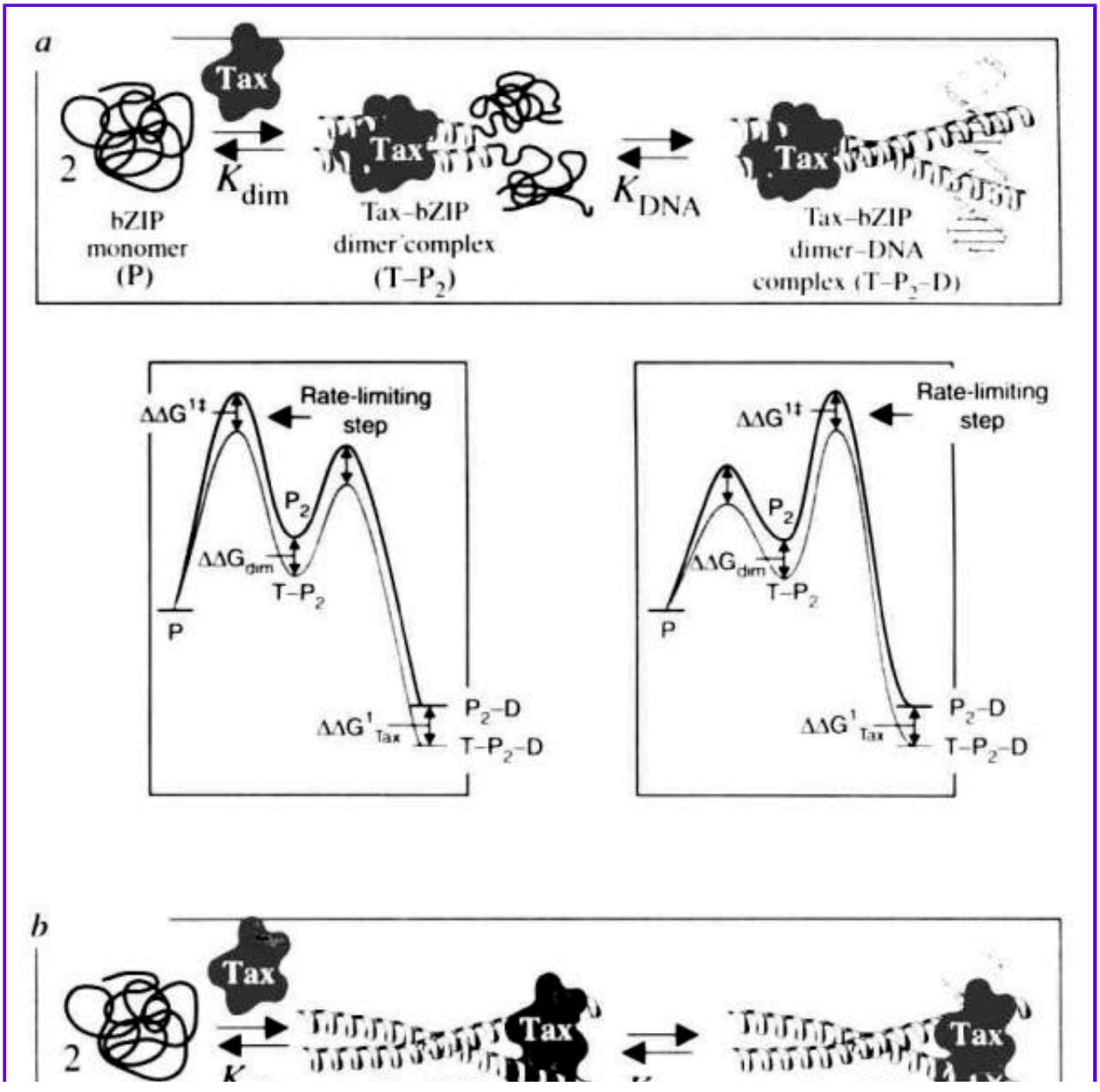
Outline

Graphics

Abstract

TAX protein activates transcription of the human T-cell leukaemia virus type I (HTLV-I) genome through three imperfect cyclic AMP-responsive element (CRE) target sites located within the viral promoter [1]. Previous work has shown that Tax interacts with the bZIP element of proteins that bind the CRE target site [2-4] to promote peptide dimerization [3,5], suggesting an association between Tax and the bZIP coiled coil. Here we show that the site of interaction with Tax is not the coiled coil, but the basic segment. This interaction increases the stability of the GCN4 bZIP dimer by 1.7 kcal mol sup -1 and the DNA affinity of the dimer by 1.9 kcal mol sup -1. The differential effect of Tax on several bZIP-DNA complexes that differ in peptide sequence or DNA conformation suggests a model for Tax action based on stabilization of a distinct DNA-bound protein structure. This model may explain how Tax interacts with transcription factors of considerable sequence diversity to alter patterns of gene expression.

Previous work has shown that Tax shifts the bZIP monomer-dimer equilibrium towards the dimer in the absence of DNA and increases the association rate, but not the dissociation rate, of the bZIP-DNA complex [3]. These data are consistent with two models (Figure 1). Model 1 invokes a direct interaction between Tax and the bZIP coiled coil that decreases the dissociation constant of the bZIP dimer (K_{dim}); model 2 invokes a direct interaction between Tax and one or more bZIP basic-spacer segments that decreases the dissociation constant of the bZIP-DNA complex, K_{DNA}, as well as K_{dim}. To distinguish model 1 from model 2, we compared the effect of Tax on two GCN4-derived peptides that differed in their ability to form a coiled coil. G_{54} contains the entire GCN4 bZIP element, whereas G^{SS} sub 29 contains two copies of the GCN4 basic-spacer segment peptide assembled into a dimer with a disulphide linkage (Figure 2) [6,7] and is therefore insensitive to a Tax-induced increase in dimerization. Model 1 predicts that Tax will have no effect on the DNA affinity of G sup SS_{29}, whereas model 2 predicts that Tax will effect G^{SS} sub 29 by an amount corresponding to the effect on DNA binding.



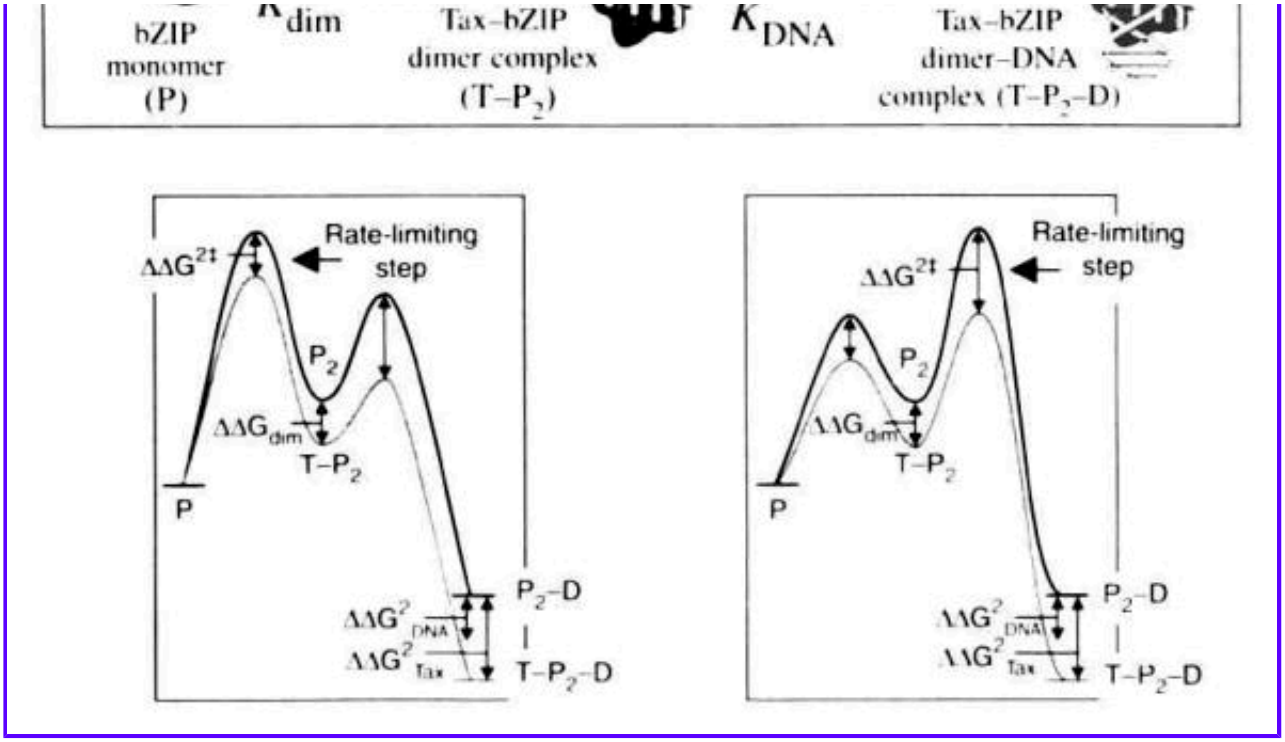


Figure 1. Two limiting models to account for the improvement in DNA-binding activity of bZIP proteins in the presence of HTLV-I Tax. Shown are reaction coordinate diagrams illustrating the relative free energies of the unfolded bZIP monomer (P), the bZIP dimer (P_2), and the bZIP-DNA complex (P_2 -D) in the presence and absence of Tax (T). The number of Tax molecules represented by T was not determined. a, In model 1, Tax interacts with the bZIP coiled coil to decrease the dissociation constant of the dimer (K_{dim}). The improvement in DNA affinity (Delta Delta G^1_{Tax}) results from a decrease in the energy of activation associated with dimerization (case 1, lower left; dimerization is rate-limiting) or from a shift in the monomer-dimer equilibrium that decreases the energy of activation associated with DNA binding (case 2, lower right; DNA binding is rate-limiting; we assume that Delta Delta G_{dim} = Delta Delta G^1 *symbol*). The absence of an effect of Tax on the dissociation rate of the bZIP-DNA complex [3] requires that the increase in apparent binding energy (Delta Delta G sup 1_{Tax}) equals the decrease in the energy of the transition state (Delta Delta G^1 *symbol*). b, In model 2, Tax interacts with the bZIP basic-spacer segment (or a pair of basic-spacer segments) to decrease the dissociation constant of the bZIP-DNA complex (K_{DNA}) as well as K_{dim}. The improvement in DNA affinity (Delta Delta G^2_{Tax}) results from a decrease in the energy of activation associated with dimerization (case 1, lower left) or DNA binding (case 2, lower right) with no decrease in the energy of activation associated with dissociation of the bZIP-DNA complex. In this case, the improvement in apparent binding energy (Delta Delta G^2_{Tax}) that accounts for the increase in affinity represents the sum of the effects of Tax on dimerization (Delta Delta G_{dim}) and DNA binding (Delta Delta G_{DNA}). Although it is unlikely that two bZIP monomers and Tax assemble into a ternary complex in a single step, the kinetic binding pathway is not known. For this reason we depict formation of the Tax-bZIP dimer complex with a single equilibrium.

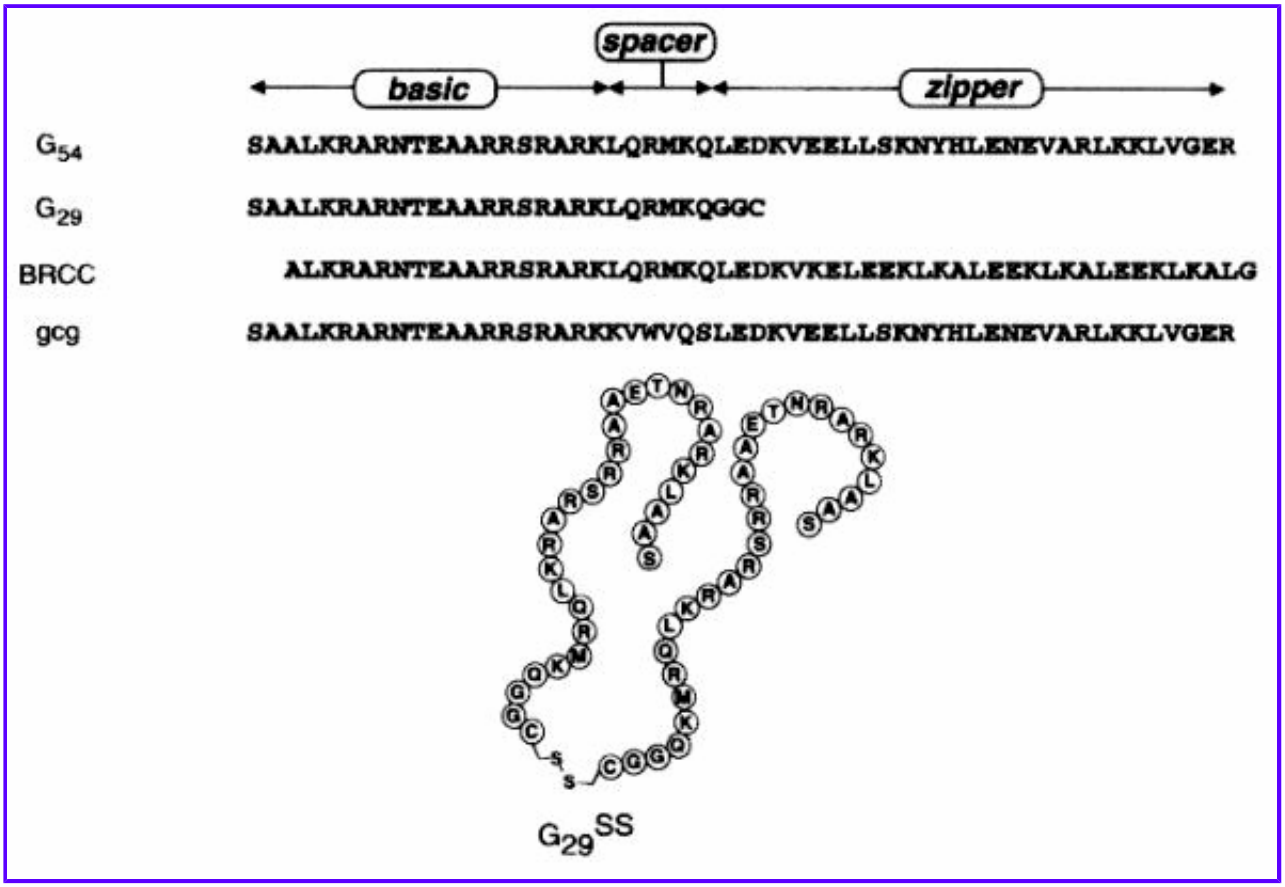


Figure 2. bZIP peptides used in this study [8,9]. The bZIP element is composed of a basic segment whose residues participate in DNA contacts [11,26] joined through a non-conserved six-residue spacer to a zipper segment responsible for protein dimerization [24].

Qualitative electrophoretic mobility shift experiments showed that Tax enhanced DNA binding by both G^{SS}_{29} and G_{54} (Figure 3). This result demonstrates an interaction between Tax and one or more bZIP basic-spacer segment(s) and provides experimental support for model 2. To determine the relative effects of Tax on dimerization (Delta Delta G_{dim}) and DNA binding (Delta Delta G^2_{DNA}), we measured the effect of Tax on the dissociation constants of the G_{54} -CRE and G^{SS}_{29} -CRE complexes (Figure 4(a)). Tax supplemented the binding energies (Delta Delta G_{obs}) of these two complexes by 3.6 and 1.9 kcal mol sup -1, respectively. Because G sub 54 was predominantly monomeric under the conditions of the DNA titration [8,9], the 3.6 kcal mol sup -1 stabilization with Tax represented the full effect on dimerization and DNA binding. Because G sup SS_{29} was a covalent dimer, the 1.9 kcal mol sup -1 stabilization with Tax represented the effect on DNA binding. Taken together, these results apportion the binding energy supplied to G_{54} by Tax (Delta Delta G^2_{Tax}) roughly equally between dimerization (1.7 kcal mol sup -1) and DNA binding (1.9 kcal mol sup -1) [5].

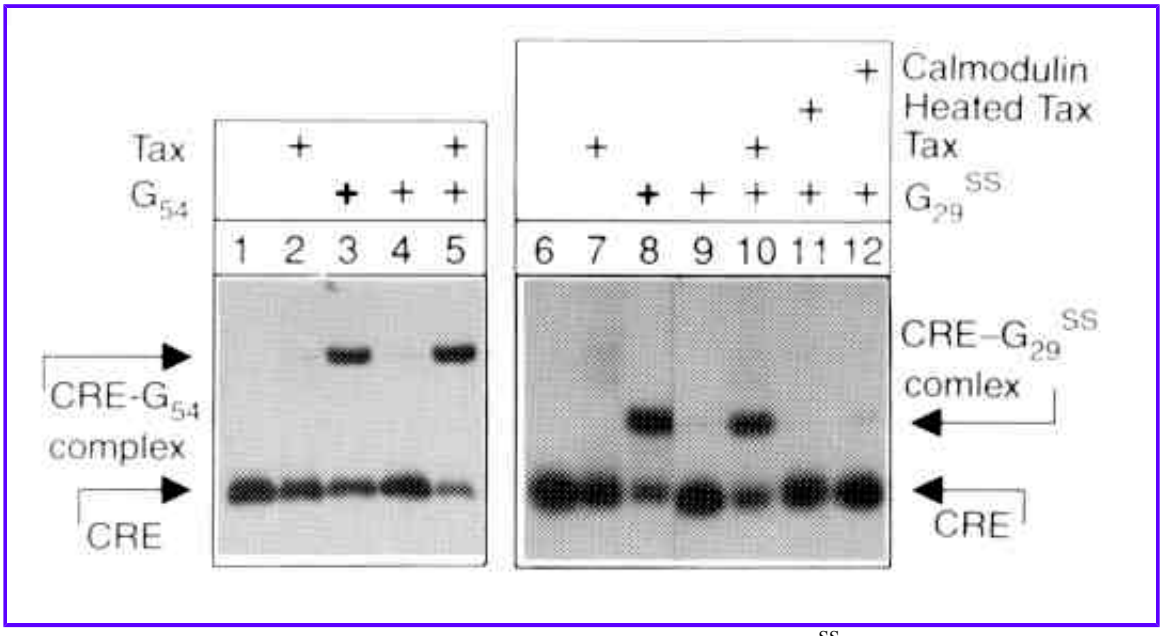


Figure 3. Qualitative analysis of the binding of a 24-base-pair CRE probe and G^{SS}_{29} [6] or G_{54} [8]. In the absence of Tax (lanes 3 and 8), G^{SS}_{29} and G_{54} showed half-maximal binding at peptide concentrations of 2 and 0.5 nM, respectively. At a concentration of 0.15 nM G_{54}, where little CRE probe was bound in the absence of Tax (lane 4), the addition of 0.3 micro Meter Tax had a significant effect on the fraction of DNA bound at equilibrium (lane 5). Similar results were obtained with G^{SS}_{29}; although little CRE probe was bound at a G^{SS}_{29} concentration of 25 pM (lane 9), a significant enhancement was observed in the presence of 0.3 micro Meter Tax (lane 10). This enhancement in binding was not seen when Tax was denatured by heating in the presence of phenanthroline (lane 11), nor when the reaction was supplemented with an equivalent concentration of calmodulin (lane 12), nor with a mixture of the molecular chaperonin GroEL, adenosine triphosphate and Magnesium^{2}+ in place of Tax. METHODS. Tax was expressed in Escherichia coli from a pTaxH_6 expression vector [25] and purified to homogeneity by Ni-chelate chromatography and FPLC (Superdex). Binding reactions contained the indicated peptide and < 50 pM of end-labelled CRE probe [6] (AGTGGAGATGACGTCATCTCGTGC) in a final reaction mixture containing 75 mM HEPES (pH 7.1), 60 mM KCl, 5 mM $MgCl_2$, 4 micro Meter $ZnSO_4$, 400 micro Meter EDTA, 0.5 micro gram micro liter sup -1 BSA, 0.5 ng micro liter sup -1 poly(dl-dC) *symbol* poly(dl-dC), 4 mM beta-mercaptoethanol, 5 ml l sup -1 Nonidet-40, 100 ml l sup -1 glycerol (final volume 10 micro liter).

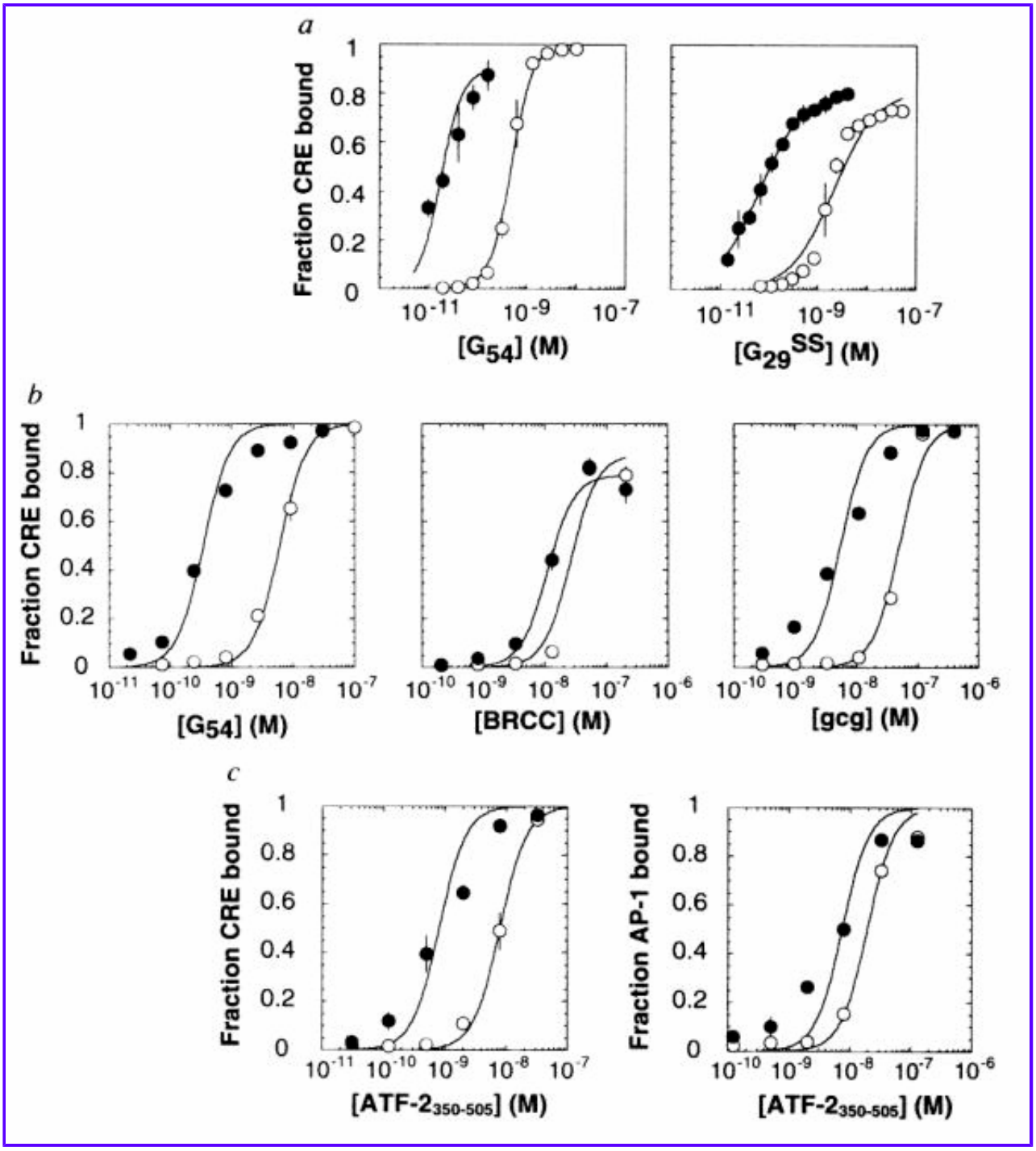


Figure 4. Quantitative electrophoretic mobility shift analysis of bZIP-DNA complex stability in the presence (filled circles) and absence (open circles) of Tax. a, Binding of the CRE probe by G_{54} and G^{SS}_{29} in the presence and absence of 5 micro Meter Tax. In the absence of Tax, the CRE probe was bound by G_{54} with an apparent dissociation constant (K_{app}) of (2.3 plus/minus 0.2) x 10 sup -19 M sup 2 and by G^{SS}_{29} with an apparent dissociation constant of (2.1 plus/minus 0.4) x 10 sup -9 M. In the presence of Tax, the dissociation constant of the G_{54} -CRE complex was (3.3 plus/minus 1.4) x 10 sup -22 M^2, whereas the dissociation constant of the G sup SS_{29} -CRE complex was (6.3 plus/minus 0.3) x 10 sup -11 M. b, Analysis of the binding of the CRE probe by G_{54}, BRCC and gcg in the presence and absence of 0.62 micro Meter Tax. Apparent dissociation constants in the absence of Tax were: G_{54} -CRE (3.8 plus/minus 0.4) x 10 sup -17 M^2; BRCC-CRE, (6.8 plus/minus 3.4) x 10 sup -16 M^2; gcg-CRE, (2.3 plus/minus 0.5) x 10 sup -15 M^2. Apparent dissociation constants in the presence of Tax were: G_{54} -CRE, (1.3 plus/minus 0.5) x 10 sup -19 M^2; BRCC-CRE, (1.2 plus/minus 0.3) x 10 sup -16 M^2; gcg-CRE, (4.6 plus/minus 1.8) x 10 sup -17 M^2. c, Analysis of the binding of the CRE and AP-1 probes by ATF-2_{350}-505 in the presence and absence of 0.62 micro Meter Tax. Apparent dissociation constants in the absence of Tax were: ATF-2 -505, (6.3 plus/minus 0.7) x 10 sup -16 M^2; ATF-2 -

505, (3.6 plus/minus 0.7) x 10 sup -17 M^2. Apparent dissociation constants in the presence of Tax were: ATF-2_{350}-505, (6.6 plus/minus 3.2) x 10 sup -19 M^2; ATF-2_{350}-505, (5.5 plus/minus 2.4) x 10 sup -17 M sup 2. METHODS. Binding reactions were performed under conditions similar to those in Figure 3 except that the final reaction mixture for b and c contained 80 mM HEPES, pH 7.1, 90 mM KCl, 7.5 mM $MgCl_2$, 6 micro Meter $ZnSO_4$, 140 ml l sup -1 glycerol, 0.7 ml l sup -1 Nonidet 40, 2.4 mM beta-mercaptoethanol, 0.1 mg ml sup -1 BSA and 0.5 ng micro liter sup -1 poly(dl-dC) *symbol* poly(dl-dC). Equilibrium dissociation constants of peptide-CRE complexes were obtained by fitting the binding data to the Langmuir equation Theta = (S) x $1/(1 + K_{app}/([peptide]_T)^n)$, where K_{app} and S are adjustable parameters (n = 1 for G^{SS}_{29}; n = 2 for G_{54}, BRCC, gcg, ATF-2_{350}-505) and Theta = fraction of DNA bound. Values for Delta G sub obs were calculated from the relationship Delta $G_{obs} = -RT \ln (1/K_d)$, where R = 0.00198 kcal mol sup -1 K sup -1 and T = 277 K. Values for Delta Delta G_{obs} are valid with the assumption that Tax does not alter the standard state, as defined by the binding conditions in the absence of Tax.

We also examined the effect of Tax on a bZIP peptide that, unlike G_{54}, formed a stable coiled-coil dimer. BRCC contained the basic and spacer segments of GCN4 fused to a zipper segment designed to exhibit high helical propensity (K_{dim} [nearly equal] 5 pM) [10]. Model 1 predicts that the DNA affinity of BRCC will be unaffected by Tax. Model 2, however, predicts that BRCC will be affected by Tax but less than by G_{54}, and this prediction was borne out by experiment: Tax enhanced the stability of the BRCC-CRE complex by 1.0 kcal mol sup -1 under conditions where the G_{54} -CRE complex was stabilized by 3.1 kcal mol sup -1 (Figure 4(b)).

To identify a set of basic-spacer segment residues important for Tax recognition, we compared the effect of Tax on a series of peptides [8,9] containing sequences from two bZIP proteins whose homology was restricted to residues essential for DNA recognition (GCN4 and CRE-BP1) [11,26]. Tax supplemented the binding energies of these peptides by between 1.0 and 3.1 kcal mol sup -1 under conditions where the binding of G_{54} was enhanced by 3.1 kcal mol sup -1 (Figure 4(b)). The effect of Tax was larger for peptides containing the GCN4 basic segment than for those containing the CRE-BP1 basic segment. However, the effect of Tax on peptides containing the GCN4 basic segment varied depending on the source of the spacer and zipper segments. For example, the CRE target-site affinity of gcg, which contained the basic and zipper segments of GCN4 linked by the spacer segment of CRE-BP1, was enhanced by 2.2 kcal mol sup -1 (Figure 2 and Figure 4(b)) (Delta Delta G_{obs} = 3.6 kcal mol sup -1). These results indicate that Tax is sensitive to sequence variation throughout the bZIP element [4].

There are many examples of transcriptional activation through CRE target sites by Tax [12], but few examples of transcriptional activation through AP-1 target sites [13-15]. These two DNA sequences differ by only a single G *symbol* C base pair and, owing to an intrinsic major groove bend in the CRE target site, they present similar recognition surfaces in the major groove [8]. To determine whether the qualitative difference in transcriptional activation through CRE and AP-1 target sites by Tax is correlated with the quantitative difference in stabilization of the corresponding bZIP-DNA complexes, we measured the effect of Tax on the CRE and AP-1 target-site affinities of the bZIP peptide ATF-2_{350}-505 [16]. Tax stabilized the ATF-2_{350}-505 -CRE complex by 2.5 kcal mol sup -1 (Delta Delta G^2_{Tax}) under conditions where it stabilized the ATF-2_{350}-505 -AP-1 complex by 1.0 kcal mol sup -1. Thus Tax increases the selectivity of ATF-2_{350}-505 for the CRE target site by 1.5 kcal mol sup -1. These results suggest that transcriptional activation through CRE target sites by Tax could be favoured because of selective stabilization of bZIP-CRE complexes. Moreover, the observation of different effects of Tax on two bZIP-DNA complexes that differ by a base pair and the conformation of DNA in the complex [8], combined with our inability to identify a set of bZIP protein side chains essential for Tax activity, support a model in which Tax stabilizes a distinct DNA-bound protein structure.

There are two general mechanisms by which Tax could improve both dimerization and DNA binding. Tax

could orient the two basic-spacer segment helices as they emerge from the dimer interface into a conformation appropriate for DNA binding. Recognition of the dimer enhances dimerization; the enhancement in DNA binding results from a reduction of the entropy required to orient the helices. Alternatively, Tax could stabilize helical structure within a single basic-spacer segment, a region that is largely unfolded in the absence of DNA [6,7,10,17]. Increased helicity within the basic-spacer segment enhances DNA binding; the lowered energetic cost of initiating helical structure enhances dimerization. Tax contains several sequences compatible with the formation of type-II polyproline helices that stabilize alpha-helical structure in certain small proteins [18,19] and may act in an intermolecular fashion to stabilize helical structure within the basic-spacer segment. The energetic models presented here provide a framework for analysing the mechanisms by which other accessory factors [20-23] interact with bZIP proteins to alter patterns of gene expression.

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