Gene regulation: **Protein escorts to the transcription ball**

J.W. Chin, J.J. Kohler, T.L. Schneider and A. Schepartz

A new way by which the potency of a eukaryotic transcription factor can be regulated has been discovered, in which nuclear factors increase the concentration of the transcription factor's active form by modulating an otherwise unfavorable equilibrium between monomeric and dimeric forms of the protein.

Address: Department of Chemistry, Yale University, New Haven, Connecticut 06511, USA.
E-mail: alanna.schepartz@yale.edu

Current Biology 1999, 9:R929–R932

0960-9822/99/$ – see front matter

It is a truth universally acknowledged that every transcriptional event relies on formation of a protein–DNA complex. Though the size of the 'preinitiation complex' for the transcription of eukaryotic protein-encoding genes seems to grow daily, and a seemingly endless number of synergistic interactions between and among upstream binding proteins continue to be identified, each component of these supramolecular complexes is connected — directly or indirectly — to a DNA-binding transcription factor bound to a specific sequence of DNA. The regulation of transcription is dependent primarily on processes that regulate either the probability that a DNA target sequence is occupied by a regulatory factor, or the potency of that factor once it is bound to its target.

Eukaryotic cells employ many mechanisms to alter the potency of DNA-binding transcription factors in response to cellular needs. Some mechanisms, such as changes in expression or cellular location, are manifested by a change in the nuclear concentration of the transcription factor and consequently in promoter occupancy. Other mechanisms of transcriptional regulation, such as phosphorylation or proteolysis, alter the activity or the availability of the factor's transcriptional activation domain, and enhance its potency independent of promoter occupancy. Recent studies have now uncovered a third way by which the potency of a eukaryotic transcription factor can be modulated. These two new examples involve nuclear proteins that modulate an otherwise unfavorable equilibrium between the monomeric and dimeric forms of a DNA-binding protein so as to increase the concentration of the transcription factor's active form.

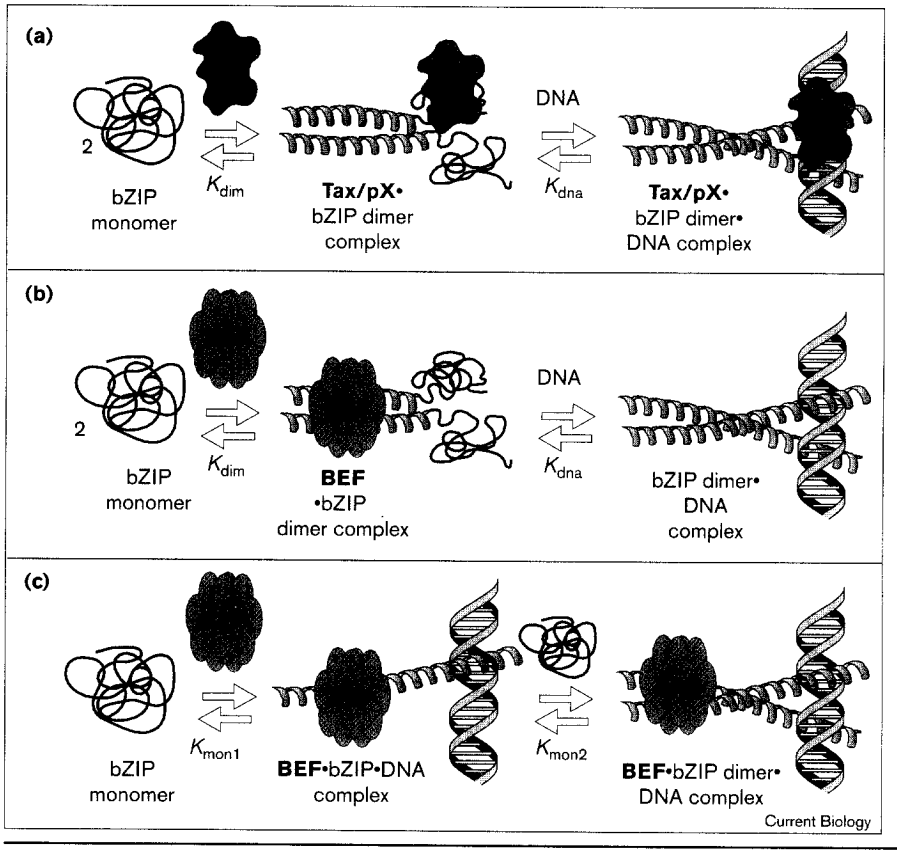
Many eukaryotic transcription factors form dimeric (2:1) protein–DNA complexes. Examples include the proteins characterised by having basic region leucine zipper (bZIP) or basic region helix–loop–helix (bHLH) DNA-binding domains, as well as many steroid hormone receptors and members of the Rel family of transcription factors. Some of these proteins exist in the nucleus as constitutive dimers [1]; others are only able to dimerize as a result of structural changes induced by DNA binding [2]. In some cases, however, the equilibrium dissociation constant of the dimer, and the estimated total protein concentration, are such that the nucleus may be home to significant levels of both monomeric and dimeric forms.

The monomer–dimer equilibrium offers an opportunity to regulate the transcriptional machinery by using auxiliary proteins that increase the equilibrium stability of the dimer and thereby the stability of the 2:1 protein–DNA complex [3]. Auxiliary proteins with such properties have been identified. For example, available data suggest that the viral coactivators Tax [4–6] and pX [7,8] interact with the DNA-contacting segments of DNA-bound bZIP proteins so as to increase bZIP dimer stability, and that this increase in stability translates into an increase in the stability of the bZIP–DNA complex. The cellular protein HMGI(Y) also enhances the level of bZIP dimerization [9]. In essence, Tax, pX and HMGI(Y) help prepare the bZIP and escort it to the DNA, remaining in attendance while the bZIP protein participates in the transcription ball (Figure 1a).

Given this precedent, it is not so surprising that a protein has been identified in HeLa cells that can increase the stability of a bZIP dimer in the absence of DNA and thereby enhance formation of the 2:1 protein–DNA complex. Virbasius *et al.* [10] have indeed discovered such a protein which, like Tax and pX, increases the affinity of a bZIP protein for DNA. This 32 kDa protein, known as 'bZIP enhancing factor' (BEF), has features in common with the viral cofactors, but displays fascinating and unexpected differences as well. Like its viral counterparts, BEF targets the DNA binding region of a bZIP protein and shifts the bZIP monomer–dimer equilibrium in the absence of DNA, promoting dimer formation. But unlike Tax and pX, which interact with the bZIP basic segment, BEF recognizes the actual dimerization element of the bZIP domain — the leucine zipper. BEF is identical to ALY, a previously identified [11] nuclear protein shown to interact with, and influence the transcriptional potency of, LEF-1 and AML-1 (which are, interestingly enough, not bZIP proteins).

The properties of BEF can be compared and contrasted with the recently discovered properties of the ubiquitous transcriptional coactivator TFIIA [12]. TFIIA binds to a

Figure 1



Three possible mechanisms of action of protein escorts for bZIP proteins. **(a)** The viral escorts Tax or pX shift the bZIP monomer–dimer equilibrium toward the dimer and remain bound to the bZIP dimer–DNA complex. The cellular escort BEF may **(b)** bind two bZIP monomers, assemble them into a coiled-coil dimer, and deliver them to the DNA; or **(c)** bind a bZIP monomer–DNA complex and facilitate dimerization on the DNA. K_{dim} and K_{dna} are the equilibrium dissociation constants of the bZIP dimer and the bZIP dimer•DNA complexes, respectively; K_{mon1} and K_{mon2} are the equilibrium dissociation constants of the bZIP monomer•DNA and bZIP dimer•DNA complexes, respectively.

dimeric form of the TATA-binding protein (TBP) that binds DNA poorly, and releases a form that binds DNA well. Like BEF, TFIIA causes a shift in a monomer–dimer equilibrium, but whereas BEF is proposed to release the dimeric form of the bZIP protein, TFIIA releases a monomeric form of TBP.

The big surprise is that BEF is not simply an escort for the bZIP protein, but also displays properties of a molecular chaperone. DNA binding by bZIP proteins is sensitive to chaperone activity — the well-studied chaperone GroEL was found to enhance DNA binding by the bZIP protein ATF-2 in an ATP-dependent manner [10]. That BEF may promote DNA binding by a bZIP protein by virtue of having chaperone-like activity is suggested by the observation that it displays an anti-aggregation activity and forms an oligomeric complex of about 14 subunits. BEF is perhaps an anomalous chaperone in two respects, though. First, neither its effect on DNA binding by bZIP proteins nor its activity in the anti-aggregation assay are dependent on energy provided by ATP hydrolysis. Second, unlike chaperones, which promote folding by avoiding off-pathway aggregation events, BEF promotes the otherwise unfavorable pathway to the properly folded molecule — the bZIP dimer.

But how does BEF work to increase the affinity of a bZIP protein for DNA? One possibility, proposed by Virbasius *et al.* [10], is that BEF binds two bZIP monomers, assembles the two monomers into a coiled-coil dimer, and releases the dimer, which can then be trapped by DNA (Figure 1b). This model is supported by the evidence that bZIP dimers are stabilized by BEF. If the preformed bZIP dimers are released into solution, as the authors propose, they have (at least) two options: they can bind DNA or dissociate into two bZIP monomers. At cellular concentrations, it is likely that DNA binding — with rate constants as high as $10^8\ M^{-1}\ s^{-1}$ [13] — will occur at a faster rate than dissociation — with rate constants between $0.05\ s^{-1}$ [13] and $0.003\ s^{-1}$ [14] — and the dimer will be trapped effectively by the available DNA. A simulation [15] of the relative flux along these two pathways, assuming a bZIP dimer concentration of 1 nM, shows that more than 70% of the bZIP dimer is trapped as the dimer–DNA complex at a DNA concentration of 5 nM, assuming that the available DNA contains the appropriate target site for formation of a sequence-specific 2:1 complex (Figure 2).

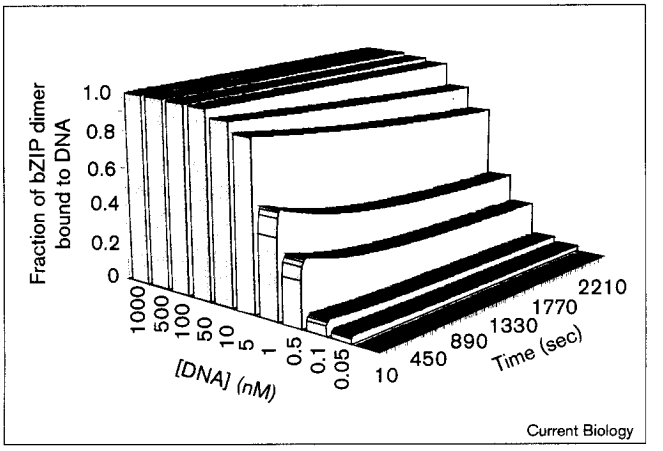
If BEF does enhance bZIP binding by this mechanism, how is the energy supplied? BEF is not an ATP-dependent

chaperone, and it remains to be determined how the bZIP dimer is released from BEF. In a slight variation of this model, BEF could remain associated with the bZIP as it binds DNA. In this case, release of BEF could be triggered by DNA binding by the bZIP dimer, or by another interaction of BEF. BEF has regions where the sequences suggest similarity to RNA recognition motifs [11]. Interactions between BEF and the nascent RNA transcript might both trigger release of BEF from the transcriptional machinery and increase the effective concentration of BEF–bZIP in transcriptionally active regions of the genome. In other words, BEF might not simply fold the bZIP into a conformation suitable for DNA binding, but also usher it into a waltz with key players at the transcription ball.

A second possible mechanism that can account for the activity of BEF invokes an alternative pathway for assembling a bZIP–DNA complex (Figure 1c). In the alternative pathway, two bZIP monomers bind DNA sequentially and isomerize into a coiled-coil dimer while bound to DNA [16]. Recent work has shown that this pathway allows the bZIP to locate its target DNA more rapidly than the pathway proceeding through a preformed dimer, because it avoids long-lived yet non-productive complexes between the bZIP dimer and non-specific DNA [13]. BEF may bind the bZIP monomer–DNA complex and aid dimer formation on the DNA, perhaps by preorganizing the α helix of the leucine zipper in an extended conformation (Figure 1c). According to this model, BEF would protect the partially unfolded zipper from proteolysis (and perhaps aggregation) and the bZIP protein would retain the kinetic specificity provided by the monomer binding pathway [13].

The identification of molecular escorts for DNA-binding proteins opens many new areas of investigation. Are these proteins essential for efficient transcriptional regulation *in vivo*? Does BEF affect bZIP activity in an obligate way, or is its activity regulated as well? Is the newly discovered activity of TFIIA regulated by interactions with upstream activators? Do other escorts assist the folding and delivery of other transcription factors? Do Tax and pX also have chaperone activity? Questions of mechanism and specificity abound: BEF lacks sequences likely to fold into a helical template for coiled-coil formation; how then is the molecular surface of BEF tailored to recognize so many different bZIP proteins, but not, apparently, Gal4 (which, like bZIP proteins, contains a coiled coil)? Does BEF actually promote folding and dimerization, or does it function like a more traditional chaperone, to avoid off-pathway folding events? And finally, does BEF, like its viral counterparts, alter the set of DNA sequences preferred by the bZIP protein? Only time will tell if these new protein escorts participate in locating (or even changing) the correct time and place of the transcription ball.

Figure 2



An illustration of how bZIP dimers released by BEF may be partitioned between a pathway in which the dimer dissociates and one in which the dimer is trapped by DNA. At low DNA concentrations very little of the preformed dimer is trapped by DNA; when the DNA concentration is increased to 5 nM, approximately 70% of the preformed dimer is trapped as the (2:1) bZIP–DNA complex.

Acknowledgements

Thanks to the Medicinal and Organic Divisions of the American Chemical Society for predoctoral fellowships to J.J.K. and T.L.S., respectively, and to the Arthur Wayland Dox Foundation and the US-UK Fulbright Commission for a predoctoral fellowship to J.W.C. Research in the authors' laboratory is supported by grants from the NIH.

References

1. Shuai K, Horvath CM, Huang LHT, Quershi SA, Cowburn D, Darnell Jr JE: **Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions.** *Cell* 1994, **76**:821-828.
2. Holmbeck SMA, Dyson HJ, Wright PE: **DNA-induced conformational changes are the basis for cooperative dimerization by the DNA-binding domain of the retinoid X receptor.** *J Mol Biol* 1998, **284**:533-539.
3. Baranger AM: **Accessory factor-bZIP-DNA interactions.** *Curr Opin Chem Biol* 1998, **2**:18-23.
4. Baranger AM, Palmer CR, Hamm MK, Giebler HA, Brauweiler A, Nyborg JK, Schepartz A: **Mechanism of DNA binding enhancement by the HTLV-I Tax protein.** *Nature* 1995, **376**:606-608.
5. Perini G, Wagner S, Green MR: **Recognition of bZIP proteins by the human T-cell leukaemia virus transactivator Tax.** *Nature* 1995, **376**:602-605.
6. Wagner S, Green MR: **HTLV-I Tax protein stimulation of DNA binding of bZIP proteins by enhancing dimerization.** *Science* 1993, **262**:395-399.
7. Palmer CR, Gegnas LD, Schepartz A: **Mechanism of DNA binding enhancement by hepatitis B virus protein pX.** *Biochemistry* 1997, **36**:15349-15355.
8. Perini G, Oetjen E, Green MR: **The hepatitis B pX protein promotes dimerization and DNA binding of cellular basic region/leucine zipper proteins by targeting the conserved basic region.** *J Biol Chem* 1999, **274**:13970-13977.
9. Du W, Maniatis T: **The high mobility group protein HMG I(Y) can stimulate or inhibit DNA binding of distinct transcription factor ATF-2 isoforms.** *Proc Natl Acad Sci USA* 1994, **91**:11318-11322.
10. Virbasius C-MA, Wagner S, Green MR: **A human nuclear-localized chaperone that regulates dimerization, DNA binding, and transcriptional activity of bZIP proteins.** *Mol Cell* 1999, **4**:219-228.
11. Bruhn L, Munnerlyn A, Grosschedl R: **ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCRα enhancer function.** *Genes Dev* 1997, **11**:640-653.

12. Coleman RA, Taggart AKP, Burma S, Chica II JJ, Pugh BF: **TFIIA regulates TBP and TFIID dimers.** *Mol Cell* 1999, **4**:451-457.
13. Kohler JJ, Metallo SJ, Schneider TL, Schepartz A: **DNA specificity enhanced by sequential binding of protein monomers.** *Proc Natl Acad Sci USA* 1999, **96**:11735-11739.
14. Zitzewitz JA, Bilsel O, Luo J, Jones BE, Matthews CR: **Probing the folding mechanism of a leucine zipper peptide by stopped-flow circular dichroism spectroscopy.** *Biochemistry* 1995, **34**:12812-12819.
15. Barshop BA, Wrenn RF, Frieden C: **Analysis of numerical methods for computer simulation of kinetic processes – development of KinSim – a flexible, portable system.** *Anal Biochem* 1983, **130**:134-145.
16. Metallo SJ, Schepartz A: **Certain bZIP peptides bind DNA sequentially and dimerize on the DNA.** *Nat Struct Biol* 1997, **4**:115-117.