Double-stranded DNA templates can induce α -helical conformation in peptides containing lysine and alanine: Functional implications for leucine zipper and helix–loop–helix transcription factors

(protein structure/ α -helix/transcriptional regulation/circular dichroism)

NEIL P. JOHNSON*, JOEL LINDSTROM, WALTER A. BAASE, AND PETER H. VON HIPPEL

Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403

Contributed by Peter H. von Hippel, February 15, 1994

ABSTRACT Transcription factors of the basic–leucine zipper and basic–helix–loop–helix families specifically recognize DNA by means of intrinsically flexible peptide domains that assume an α -helical conformation upon binding to target DNA sequences. We have investigated the nonspecific interactions that underlie specific DNA recognition. Circular dichroism measurements showed that 20-bp double-stranded DNA oligonucleotides can act as templates to promote random coil $\rightarrow$ α -helix transitions in short peptides containing alanine and lysine. This conformational change takes place without altering the structure of the DNA, and neither specific peptide–DNA contacts nor cooperative interactions between peptides are necessary. The conformational change does require (i) double-stranded (but not single-stranded) oligodeoxynucleotides in either the B or the B' conformation and (ii) peptides that can form positively charged amphipathic α -helices. In 10 mM Na_2HPO_4 (pH 7.5; 10°C), the excess free-energy contribution of the DNA template to the stability of the α -helical form of the oligopeptides tested was $\Delta G_{\text{ex}} = -0.15 (\pm 0.07)$ kcal/mol per lysine residue. The implications of these results for the thermodynamics and kinetics of DNA target site selection by basic–leucine zipper and basic–helix–loop–helix regulatory proteins are discussed.

Eukaryotic regulatory proteins of the basic–leucine zipper (bZIP) and basic–helix–loop–helix (bHLH) families bind as dimers to symmetric DNA sites. Each of the monomers contains a 60- to 100-aa sequence composed of two domains, both of which are required for efficient specific complex formation. One, the HLH or the ZIP domain, provides the protein–protein interactions involved in dimer formation. The second selectively binds the DNA target sequence. This DNA-binding motif generally contains several basic amino acid residues and has a flexible conformation in the absence of DNA. On binding to the major groove of the specific DNA target sequence it forms an amphipathic α -helix (i.e., an α -helix with all of the positively charged residues located on one side), thereby introducing sufficient topological constraints to selectively recognize complementary hydrogen bonding and van der Waals sites within the DNA double helix (1–10).

Specific DNA-binding proteins distinguish their target sequences from a vast number of competing nonspecific sites by means of large free energy differences between specific and nonspecific protein–DNA complexes. Rigid DNA-binding domains, such as the helix–turn–helix (HTH) motif, drive selective recognition by imposing hydrogen-bonding interactions that destabilize nonspecific protein–DNA complexes relative to those with the correct target sequence (11–13). Proteins with flexible DNA-binding domains (such

as bZIP and bHLH) might be expected to avoid unfavorable interactions and consequently to bind more tightly at nonspecific sites than proteins with rigid DNA-binding motifs. Furthermore, binding free energy must be used to drive unstructured binding domains into the bound conformation; if this structure-forming reaction occurs only during binding at the regulatory site, then specific complexes would be selectively destabilized relative to nonspecific complexes. Hence, specific recognition of DNA target sites by flexible binding domains would be significantly more thermodynamically “expensive” than recognition by initially structured motifs, in that a larger amount of the protein would have to be synthesized and bound at nonspecific DNA sites to maintain binding equilibrium at the specific sites (12–14). Finally, the rate of specific target location by a regulatory protein might also be significantly decreased if the binding domain of the protein can assume its docking and recognition conformation only on arrival at the specific DNA target sequence (15).

In order to understand the thermodynamic and kinetic bases of specific binding by bZIP and bHLH transcription factors, it is important to know whether their flexible DNA-binding motifs are also driven into an α -helix when the protein is bound at nonspecific DNA sites. To this end we asked whether DNA-induced α -helix formation must be stabilized with the assistance of a specific complementary hydrogen-bonding docking matrix or whether it could occur at nonspecific peptide–DNA interactions. We show here that small peptides with appropriately placed alanine and lysine residues do indeed have the potential to form α -helices by interacting nonspecifically with double-stranded DNA, suggesting that helical DNA-binding motifs may be generally present in nonspecific as well as specific complexes between bZIP or bHLH transcription factors and DNA. This result has important thermodynamic, kinetic, and structural consequences for transcriptional regulation which are considered in the *Discussion*.

MATERIALS AND METHODS

Preparation and Characterization of Oligopeptides and Oligonucleotides. The peptides designated A2K2 and A2KA3K (Fig. 1) were synthesized on an Applied Biosystems model 430A apparatus in the University of Oregon Biotechnology Laboratory by means of standard *tert*-butoxycarbonyl chemistry and were purified by reverse-phase HPLC on a C_{18} resin with elution by a 0–70% acetonitrile gradient in 0.1% trifluoroacetic acid. Purity was evaluated by amino acid se-

Abbreviations: bZIP, basic–leucine zipper; bHLH, basic–helix–loop–helix; HTH, helix–turn–helix.

*Permanent address: Laboratoire de Pharmacologie et de Toxicologie Fondamentales du Centre National de la Recherche Scientifique, UPR 8221, 205 route de Narbonne, 31077 Toulouse, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

these expectations we find that the amplitude of the CD spectra near 220 nm, for an equimolar mixture of A2KA3K and dsAT from which the spectrum of dsAT has been subtracted (lower curves in Fig. 2), is approximately doubled relative to the spectrum of A2KA3K alone (upper curves). Similar increases in CD amplitudes were also observed for complexes of A2KA3K with (dA₂₀)(dT₂₀), and for complexes of A2K2 with both of these oligonucleotides (Fig. 2b). In contrast, equimolar mixtures of (dA₂₀)(dT₂₀) or dsAT with A4K and AK displayed CD spectra that were identical to the sum of the component spectra (data not shown).

The peptides that we have used do not contribute to the molar ellipticity at 260–300 nm, where optical activity reflects oligonucleotide base stacking. No intensity was observed in the CD difference spectrum at these wavelengths for any of our peptide/oligonucleotide mixtures (Fig. 2), indicating that DNA base stacking was not perturbed by peptide binding. Instead, these CD difference spectra are characteristic of small alanine/lysine-containing peptides in an α -helical conformation (20). Although we cannot rule out a contribution from the oligonucleotide to the 200- to 240-nm region of the CD spectrum, it seems reasonable, judging from the shapes and intensities of the difference spectra, to assign the spectral changes observed in Fig. 2 to an increase in the α -helical content of the peptides.

To further investigate this helix-induction phenomenon, we titrated oligonucleotides with peptides and followed the molar ellipticity at 223 nm ($[\theta]_{223}$) as a function of total peptide concentration (Fig. 3). The CD signal of the oligonucleotide alone, corrected for dilution, has been subtracted from these data. Linear titration curves were obtained when peptides were added to solutions containing buffer only (Fig. 3a), indicating that helix formation was not stabilized by associations between peptide molecules. Molar ellipticities calculated from the slope of these titration curves were the same as those obtained from the CD spectra of the peptides alone. However, when the peptides were titrated into some of the oligonucleotide solutions, the values of $[\theta]_{223}$ obtained fell below the straight line obtained for titration into buffer alone (Fig. 3 b–d). Control CD spectra showed that the additional negative ellipticity observed at 223 nm corresponded to a CD difference spectrum similar to that of Fig. 2. Thus we interpret the changes in the slopes of the titration curves of Fig. 3 in terms of DNA-induced increases in the α -helix content of certain peptides. Replicate determinations

FIG. 2. CD spectra and difference spectra of the alanine/lysine peptides in the presence and absence of various DNA oligonucleotides. (a) Spectrum of 1 μ M A2KA3K alone ($\bullet$) and as a difference spectrum of 1 μ M dsAT and equimolar A2KA3K from which the spectrum of 1 μ M dsAT alone has been subtracted ($\blacksquare$). (b) Spectrum of 0.8 μ M A2K2 alone ($\bullet$) and as a difference spectrum of 0.8 μ M (dA₂₀)(dT₂₀) and equimolar A2K2 from which the spectrum of 0.8 μ M (dA₂₀)(dT₂₀) alone has been subtracted ($\blacksquare$). Experiments were performed at pH 7.5 and 10°C in the presence of either 50 mM Na₂HPO₄ (a) or 5 mM Na₂HPO₄/50 mM NaCl (b).

FIG. 3. Typical data for the titration of the indicated oligonucleotides or buffer only with A2K2 ($\bullet$, top curve, except for d where this peptide is not shown; see text), A4K ($\blacksquare$, bottom curve), or A2KA3K ($\blacktriangle$, middle curve) in 10 mM Na₂HPO₄ (pH 7.5, 10°C). θ_{223} , ellipticity at 223 nm in a 1-cm-path-length cell. Nucleotide concentrations were 2 μ M single-stranded (ssAT) or double-stranded [dsAT and (dA₂₀)(dT₂₀)] oligonucleotide templates.

of the initial slope from such titrations were used to compute the α -helix content of each peptide in the presence and absence of the various oligonucleotide templates (Table 1).

The binding of A2KA3K to ssAT did not change the helix content of the peptide compared with buffer (40% and 34%, respectively; Table 1). In contrast, titration of dsAT or (dA₂₀)(dT₂₀) with this peptide resulted in initial slopes less than those obtained in the equivalent buffer titration (60% α -helical content; Table 1). Thus, A2KA3K became more α -helical in the presence of double-stranded DNA (but not in the presence of single-stranded DNA). Similar analyses showed that the α -helix content of A2K2 increased from 10% to 33% in the presence of dsAT and (dA₂₀)(dT₂₀) (Table 1). Precipitation occurred during titration of ssAT with A2K2 (data not shown). Prior to precipitation the amplitude of the CD spectrum of ssAT decreased at its 270-nm peak and increased at its 250-nm trough, indicating that this peptide further unstacked the single-stranded DNA. This behavior was not observed on the addition of A2K2 to double-stranded DNA.

Identical titration curves were obtained for the addition of A4K to buffer or to oligonucleotide solutions (bottom trace in all panels of Fig. 3). The CD signal decreased linearly as a function of the concentration of added peptide, and the slope indicated that this peptide remained about 50% helical in the presence or absence of oligonucleotides (Table 1).

Table 1. α -Helix content of peptides with and without DNA oligonucleotides in 10 mM Na₂HPO₄ (pH 7.5, 10°C)

Peptide	% helix (mean $\pm$ range)			
	In buffer	With dsAT	With ssAT	With (dA ₂₀)(dT ₂₀)
A2K2	10.2 $\pm$ 0.6	33.3 $\pm$ 4.0	Pptn	31.0 $\pm$ 2.4
A2KA3K	33.7 $\pm$ 3.0	60.9 $\pm$ 9.4	39.8 $\pm$ 1.5	57.5 $\pm$ 3.3
A4K	46.9 $\pm$ 2.7	52.2 $\pm$ 3.7	50.8 $\pm$ 0.8	51.6 $\pm$ 1.6

Pptn, precipitation.

DISCUSSION

We have shown that, at low salt concentrations and temperatures, double-stranded (but not single-stranded) DNA templates can promote α -helix formation in the oligopeptides A2K2 and A2KA3K (Table 1). In contrast, none of the DNA templates we tested was able to induce an increase in the α -helical content of the peptide A4K. Clearly this does not reflect conformational constraints within A4K, since this peptide becomes 80% α -helical when charge repulsion between lysine residues is screened by addition of 1 M NaCl, and becomes fully α -helical in trifluoroethanol (20). The lysine residues of A4K are separated by four alanine residues and thus A4K cannot form an amphipathic α -helix, whereas A2K2 and A2KA3K do form such helices. The bZIP DNA-binding domain of the GCN4 dimer, a positively charged peptide that has evolved for specific binding and carries residues other than alanine and lysine, can also assume an amphipathic, partially α -helical conformation on nonspecific templates (7). These results suggest that the potential for DNA-directed α -helix induction is general for positively charged peptides that can form amphipathic α -helices. The ability to drive helix formation in these peptides does not appear to be very sensitive to the structure or the flexibility of the double-helical DNA template, at least with the oligomers tested (Table 1). Likewise, the unchanged CD spectra in the vicinity of 260 nm (Fig. 2) indicate that peptide helix induction does not alter the conformations of the oligonucleotide partners.

We have used a simple two-state thermodynamic model to estimate the contribution of the double-stranded oligonucleotide templates to the free energy of the coil $\rightleftharpoons$ helix transition of the peptides that we have studied:[†]

$$cD \rightleftharpoons c + D \rightleftharpoons h + D \rightleftharpoons hD. \quad [2]$$

In this model $K_1 = [h]/[c]$, $K_2 = [hD]/[h][D]$, and $K_3 = [cD]/[c][D]$, with c representing a peptide molecule in the random coil (unfolded) form, h a peptide molecule in the α -helical form, and D a double-stranded DNA template molecule. cD and hD represent complexes of DNA templates with peptides in the coil and helical forms, respectively. These equilibria describe the binding of peptide to DNA in the limit at which a single peptide interacts with a single DNA template molecule to form a 1:1 complex. Our results show that double-stranded DNA templates preferentially bind the peptide in the α -helical conformation and shift the equilibria of Eq. 2 to the right.

Based on Eq. 2, the overall equilibrium between peptide in the coil and helix forms can be written:

$$K = ([h] + [hD])/([c] + [cD]). \quad [3]$$

In the absence of DNA, K becomes K_1 , which may be calculated from the measured helix content of the peptide free in solution (Table 2). K is determined from the helix content of the peptide in the presence of excess DNA. From the equilibrium binding constants we may write

[†]Clearly, as Baldwin and coworkers (24) have shown, such a model is not adequate, in general, to describe helix $\rightleftharpoons$ coil transitions in solution in short peptides of this sort. Distributions of partially helical molecules are actually obtained at the midpoint of the transition, and the use of a two-state model can significantly underestimate helix content under some conditions. Thus the approximations involved in using the two-state model must be borne in mind in evaluating our quantitative results. Nevertheless, the two-state model may be reasonably appropriate in the situation described here, since a peptide bound to a double-stranded DNA template is more likely to be primarily helical than the same peptide free in solution.

$$K = K_1(1 + K_2[D])/(1 + K[D]), \quad [4]$$

and the free energy of the coil $\rightleftharpoons$ helix transition ($\Delta G = -RT\ln K$) is

$$\Delta G = \Delta G(0) + \Delta G_{\text{ex}}(D), \quad [5]$$

where $\Delta G(0) = -RT\ln K_1$ is the free energy of helix formation in the absence of DNA, and $\Delta G_{\text{ex}}(D) = -RT\ln\{(1 + K_2[D])/(1 + K[D])\}$ is the excess free energy of the coil $\rightarrow$ helix transition due to DNA binding. The contribution of the DNA template to the free energy of helix formation is then

$$\Delta G_{\text{ex}} = -RT\ln(K/K_1). \quad [6]$$

Hence the double-stranded oligonucleotide template contributes a standard free energy (ΔG_{ex}) of approximately $-0.8 (\pm 0.1)$ kcal/mol of oligopeptide to the helix formation of A2K2 and approximately $-0.6 (\pm 0.3)$ kcal/mol of oligopeptide to the helix formation of A2KA3K (Table 2). This corresponds to an average ΔG_{ex} of $-0.15 (\pm 0.07)$ kcal/mol for each lysine residue in the peptide. We have observed (data not shown) that helix induction in the A2K2 peptide is suppressed by 0.1 M NaCl, as expected for a stabilization mechanism that is primarily based on electrostatic interactions between properly placed lysine residues and DNA phosphate groups.

From Eq. 4, $K/K_1 = K_2/K_3$ for $K_3[D] \gg 1$. Since at high DNA/peptide ratios $[D]$ is the total nucleotide template concentration (2 μ M double-stranded oligonucleotide), K_3 must be $\gg 5 \times 10^5 \text{ M}^{-1}$. The peptides that we have examined in this study contained four or six lysine residues. Consequently, K_3 , the equilibrium constant for binding a peptide to DNA in the random coil form at 10°C in 10 mM Na_2HPO_4 (pH 7.5), can be estimated, to a first approximation, as 6×10^9 per mol of oligonucleotide from the binding constant of (Lys)₅ to DNA under equivalent solvent conditions (25). If this estimate is correct, the helix $\rightleftharpoons$ coil equilibrium in the presence and in the absence of DNA is simply related to the relative binding of h and c to DNA, $K/K_1 = K_2/K_3$, and we can estimate that the binding constant of dsAT to the peptides A2K2 and A2KA3K in the α -helical conformation is about 4 times greater than that for the binding of dsAT to the equivalent random-coil peptides.

The free energy of binding of our peptides in the random-coil form to DNA can be estimated as -13 kcal/mol of oligonucleotide from the binding constant of (Lys)₅ to DNA under our experimental conditions (25). In contrast, the contribution of the double-stranded DNA templates to the free energy of α -helix formation for these peptides is quite small: approximately -0.75 kcal/mol for a peptide carrying five lysine residues that can form an amphipathic α -helix. These relative free energies are consistent with genetic data (27) suggesting that the flexibility of the DNA-binding domain may be important in specific recognition of transcription factors of this type. The need to accommodate sterically demanding peptide-DNA interactions, such as hydrogen-bond formation, could easily provoke minor shifts in the position and orientation of the peptide within the major groove of DNA, allowing similar positively charged DNA-reading heads to recognize different members of a family of related regulatory DNA sites.

Table 2. Thermodynamic parameters for the coil $\rightleftharpoons$ helix equilibrium in the presence and absence of dsAT

Parameter	A2K2	A3KA2K	A4K
In buffer (K_1)	0.114 ± 0.007	0.51 ± 0.07	0.88 ± 0.10
With dsAT (K)	0.50 ± 0.09	1.56 ± 0.81	1.09 ± 0.18
ΔG_{ex} , kcal/mol	-0.8 ± 0.1	-0.6 ± 0.3	0

The induction of α -helical conformations by nonspecific DNA sequences might be thermodynamically important for specific binding of regulatory proteins in at least two ways. (i) If helix formation occurs only upon contact with the DNA target sequence, the binding free energy diverted to this purpose will decrease specific binding affinity relative to nonspecific binding affinity. On the other hand, if the binding domain assumes a helical conformation at both specific and nonspecific sites, this extra cost of specific binding is avoided. (ii) In the HTH proteins, unfavorable interactions between the rigid DNA-binding domain and DNA at nonspecific sites cannot be avoided and the resulting unfavorable free energy drives specific binding of the protein (11, 14). In contrast, a flexible DNA-binding motif might decrease specificity by avoiding these unfavorable interactions at nonspecific sites and minimizing their destabilizing impact. DNA-induced α -helix formation at the protein reading head may permit bZIP and bHLH DNA proteins to resolve this apparent contradiction between flexibility and specificity by introducing topological constraints that prevent the DNA-binding domain from avoiding unfavorable contacts at nonspecific DNA sequences.

Finally, the presence of the same conformation at nonspecific and specific sites would permit the retention of the main structural elements of the recognition domains in a scanning or sliding approach to the specific target site along the DNA. The ability to slide, dock, and recognize on moving from nonspecific to specific sites without major conformational rearrangements should speed binding to the regulatory target sequence (15).

What evolutionary advantages might lead to the selection of transcription factors with flexible DNA-binding domains? The thermodynamic bases for specific binding would remain unchanged if binding to both specific and nonspecific sites required the induction of α -helix. However, interactions with both types of sites would be destabilized by the presence of an unstructured binding domain in the unbound protein, thereby increasing the free protein concentration required to maintain binding equilibrium. For example, if the free-energy cost of putting the recognition domain into an α -helix were 1 kcal/mol (a reasonable value judging from our results with model peptides), then 5 times more "free" protein would have to be available in solution for a protein with a flexible binding domain than for a peptide with a rigid DNA-binding site. Increased concentrations of free protein could significantly drive intermolecular associations, such as the formation of homo- and heterodimers by bZIP and bHLH proteins that are important for their regulation (26). These interactions would be less accessible to an equivalent transcription factor with a rigid DNA-binding domain.

It may be significant for this argument that regulatory proteins with flexible DNA-binding domains are more common in eukaryotic than in prokaryotic cells. Transcriptional activation (regulated by the formation of multiprotein complexes) is also more common in eukaryotes. Transcriptional activation mechanisms generally allow more complex regulation of gene expression, and the resulting increase in adaptive cellular response may account, in part, for the evolution of regulatory proteins with flexible DNA-binding domains in higher organisms.

We thank Deborah McMillan of the University of Oregon Bio-

technology Laboratory for synthesizing some of the oligopeptides used in this study and Robert Baldwin, S. Padmanabhan, and Warner Peticolas for supplying additional peptides (see *Materials and Methods*). We are very grateful to Robert Baldwin and John Schellman for helpful discussions. These studies were supported in part by U.S. Public Health Service Research Grants GM15792 and GM29158 (to P.H.v.H.) and by a grant to the Institute of Molecular Biology from the Lucille P. Markey Charitable Trust. P.H.v.H. is an American Cancer Society Research Professor of Chemistry. N.P.J. gratefully acknowledges additional support from the Centre Nationale de la Recherche Scientifique.

- Vinson, C. R., Sigler, P. B. & McKnight, S. L. (1989) *Science* **246**, 911–916.
- Talanian, R. V., McKnight, C. J. & Kim, P. S. (1990) *Science* **249**, 769–771.
- O'Neil, K. T., Hoess, R. H. & Degrado, W. F. (1990) *Science* **249**, 774–778.
- Weiss, M. A., Ellenberger, T., Wobbe, C. R., Lee, J. P., Harrison, S. C. & Struhl, K. (1990) *Nature (London)* **347**, 575–578.
- Anthony-Cahill, S. J., Benfield, P. A., Fairman, R., Wasserman, Z. R., Brenner, S. L., Stafford, W. F., III, Altenbach, C., Hubbell, W. L. & DeGrado, W. F. (1992) *Science* **255**, 979–983.
- Saudek, V., Pasley, H. S., Gibson, T., Gausepohl, H., Frank, R. & Pastore, A. (1991) *Biochemistry* **30**, 1310–1317.
- O'Neil, K. T., Shuman, J. D., Ampe, C. & DeGrado, W. F. (1991) *Biochemistry* **30**, 9030–9034.
- Ellenberger, T. E., Brandt, C. J., Struhl, K. & Harrison, S. C. (1992) *Cell* **71**, 1223–1237.
- Ferré-D'Amaré, A. R., Prendergast, G. C., Ziff, E. B. & Burley, S. K. (1993) *Nature (London)* **363**, 38–45.
- König, P. & Richmond, T. J. (1993) *J. Mol. Biol.* **223**, 139–154.
- von Hippel, P. H. & Berg, O. G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1608–1612.
- Berg, O. G. & von Hippel, P. H. (1987) *J. Mol. Biol.* **193**, 723–750.
- Berg, O. G. & von Hippel, P. H. (1988) *J. Mol. Biol.* **200**, 700–723.
- von Hippel, P. H., Revzin, A., Gross, C. A. & Wang, A. C. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4808–4812.
- von Hippel, P. H. & Berg, O. G. (1989) *J. Biol. Chem.* **264**, 675–678.
- Brandts, J. F. & Kaplan, L. J. (1973) *Biochemistry* **12**, 2011–2024.
- Chakrabarty, A., Kortemme, T., Padmanabhan, S. & Baldwin, R. L. (1993) *Biochemistry* **32**, 5560–5565.
- Cantor, C. R., Warshaw, M. M. & Shapiro, H. (1970) *Biopolymers* **9**, 1059–1077.
- Dao-pin, S., Baase, W. A. & Matthews, B. W. (1990) *Proteins: Struct. Funct. Genet.* **7**, 198–204.
- Padmanabhan, S., Marqusee, S., Ridgeway, T., Laue, T. M. & Baldwin, R. L. (1990) *Nature (London)* **344**, 268–270.
- Chan, S. S., Breslauer, K. J., Hogan, M. E., Kessler, D. J., Austin, R. H., Ojemann, J., Passner, J. M. & Wiles, N. C. (1990) *Biochemistry* **29**, 6161–6171.
- Yoon, C., Privé, G. G., Goodsell, D. S. & Dickerson, R. E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6332–6336.
- Schlotz, J. M. & Baldwin, R. L. (1992) *Annu. Rev. Biophys. Biomol. Struct.* **21**, 95–118.
- Chakrabarty, A., Schellman, J. A. & Baldwin, R. L. (1991) *Nature (London)* **351**, 586–588.
- Lohman, T. M., DeHaseth, P. L. & Record, T. M., Jr. (1980) *Biochemistry* **19**, 3522–3530.
- Lamb, F. & McKnight, S. L. (1991) *Trends Biochem. Sci.* **16**, 417–422.
- Kim, J., Tzamarias, D., Ellenberger, T., Harrison, S. C. & Struhl, K. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4513–4517.