Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7387–7391, October 1989
Biochemistry

Modulation of the stability of a gene-regulatory protein dimer by DNA and cAMP

(catabolite activator protein/*Escherichia coli*/affinity chromatography/subunit exchange/ligand binding)

ABRAHAM M. BROWN* AND DONALD M. CROTHERS

Department of Chemistry, Yale University, New Haven, CT 06511

Contributed by Donald M. Crothers, July 3, 1989

ABSTRACT **We describe an experimental approach to the measurement of protein subunit exchange in which biotinylated subunits mediate attachment of ^{35}S-labeled subunits to a streptavidin column as a result of the exchange process. Application of the method to *Escherichia coli* catabolite activator protein (CAP) revealed that in the absence of cAMP, the dimerization equilibrium constant is 3×10^{10} M^{-1}, with a dimer lifetime of 300 min. Exchange of CAP subunits is accelerated at least 1000-fold by the presence of nonspecific DNA, under low ionic strength conditions. Catalysis of exchange also occurs at physiological ionic conditions. In contrast, physiological concentrations of cAMP stabilize CAP with respect to subunit exchange in either the presence or the absence of DNA. We discuss the functional implications of monomerization of gene-regulatory proteins resulting from kinetic and thermodynamic lability of their dimers.**

During the past several years there has been rapid growth of structural information about gene-regulatory proteins, particularly prokaryotic helix–turn–helix proteins (1, 2). X-ray crystal structures have been reported for at least two ligand-regulated proteins, catabolite activator protein (CAP) and *trp* repressor (1, 3, 4), and for several other regulatory proteins (5, 6). These molecules have in common a homodimeric composition, generally thought to be an efficient motif by which to gain greater binding affinity and specificity for their respective pseudosymmetric DNA binding sequences (7). Subunit interchange of dimeric regulatory proteins has been postulated in order to explain phenomena ranging from transcriptional regulation by heterodimers (8, 9) to catalysis by DNA of the dissociation of a protein–DNA complex by stepwise removal of the monomers (10).

CAP is a homodimeric protein at physiological ionic strength. The ability of CAP to stimulate DNA-dependent transcription by *Escherichia coli* RNA polymerase is cAMP-dependent. In the absence of polymerase, cAMP stimulates sequence-specific binding of CAP to the DNA region upstream of the promoter; without cAMP only weaker, nonspecific DNA binding is observed. The dimer interaction in CAP is through hydrophobic surfaces of identical subunits, and each of the two cAMP binding sites are composed of elements of both subunits (3). Gel electrophoresis studies have indicated that the dissociation of specific CAP–promoter complexes displays a second-order dependence on competing DNA concentration. One of the mechanisms proposed involves sequential removal of monomers from the specific complex by nonspecific competitor DNA (10).

In this article we describe a direct assay to test this hypothesis, employing an approach in which biotinylated CAP subunits act to retain ^{35}S-labeled subunits to a streptavidin column as a result of subunit exchange. The assay was employed to assess the effect of natural ligands such as cAMP and DNA. We find that DNA alone destabilizes the CAP dimer, whereas cAMP has a stabilizing effect. These results lead us to propose that cAMP regulates the gene-activating activity of CAP by modulating the equilibrium between monomer and dimer forms.

MATERIALS AND METHODS

Protein Preparation. Unlabeled CAP was purified from overproducing *Escherichia coli* cells by affinity chromatography on cAMP-agarose (11). Radiolabeled CAP was prepared from methionine-requiring, overproducing cells grown in [^{35}S]methionine-supplemented medium, by a modification of the affinity method. All protein preparations were stored at 4°C in 50 mM potassium phosphate, pH 7/0.5 M KCl/0.2 mM EDTA/0.2 mM dithiothreitol/5% (vol/vol) glycerol (storage buffer). Biotinylated CAP was prepared by reacting CAP with sulfosuccinimidyl 6-(biotinamido)hexanoate (Pierce). In order to retain specific binding activity, critical residues were protected by binding CAP to DNA-cellulose (Pharmacia P-L Biochemicals) prior to chemical modification. CAP (2 mg) was bound to 1.2 ml of DNA-cellulose (1.2 mg/ml) in 20 mM Hepes, pH 8/1 mM EDTA/5% glycerol. The bound protein was biotinylated by passing 3 ml of 0.6 mM biotin reagent in Hepes buffer through the column at room temperature. After 1 hr the column was washed with 5 ml of 10 mM Tris/HCl, pH 8/1 mM EDTA to quench unreacted reagent and protein was eluted with storage buffer. cAMP-dependent specific DNA binding activity of biotinyl-CAP was identical to that of unreacted CAP, as determined by gel electrophoresis assay (10). Proteins were electrophoresed in SDS/15% polyacrylamide gels (75:1 acrylamide/*N*,*N*′-methylenebisacrylamide weight ratio) and silver-stained (12).

Subunit Exchange Assay. Five milliliters of streptavidin-agarose (from BRL) was batch-washed with four 25-ml volumes of binding buffer (10 mM Tris hydrochloride/NaOH, pH 8/1 mM EDTA/50 mM NaCl/0.05% Tween-80/10% glycerol). The soft-packed resin was mixed with 5 ml of binding buffer and 0.9 mg of CAP to block nonspecific binding. Sixty microliters of the resin mixture was placed in each 1.5-ml microcentrifuge tube. Generally, 100-μl aliquots of a reaction mixture were collected and added to each assay tube; smaller aliquots were adjusted to 100 μl final volume. Binding was allowed to proceed for 7 min with four or five rounds of gentle mixing, and the resin was then pelleted by centrifugation and the supernatant was collected. The resin was washed three times by addition of 150 μl of binding buffer, mixing, and pelleting. All supernatants were placed directly into a single glass scintillation vial. Bound radiolabeled subunits were released by addition of 200 μl of elution buffer (binding buffer plus 0.5% SDS). After mixing and

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.

Abbreviation: CAP, catabolite activator protein.

*Present address: Department of Biological Sciences, Columbia University, New York, NY 10027.

incubation for 5 min the supernatant was placed in a scintillation vial. A second volume of elution buffer was added and both resin and buffer were transferred to the vial. Eight milliliters of Packard Opti-fluor was added to each vial and scintillation counting was done in a Packard Tri-carb. Values used were either an average of two or three 2-min accumulations or one 5-min accumulation with background subtraction.

RESULTS

Subunit Exchange Assay. In our technique, separate chemically biotinylated and radiolabeled preparations of the same protein are mixed together and incubated under various solution conditions (Fig. 1). The mixture is then bound to streptavidin-agarose, which retains biotinylated protein. Radiolabeled subunits will be retained by the affinity resin only if they are tightly associated with biotinylated subunits; nonspecific retention is reduced to a few percent of added radioactivity by blockage of the column with nonradioactive, nonbiotinylated CAP and by use of the nonionic detergent Tween 80 in the binding buffer (13). Retention of radioactivity is due to the presence of mixed dimers and therefore reflects subunit exchange. Nonbiotinylated subunits retained on the column are then released by brief treatment with SDS. The extent of subunit exchange is quantitated by calculating the ratio of label released by SDS treatment to total radiolabel. The simplest application of this assay is to follow the exchange kinetics of dimeric CAP in solution. Formation of transient radiolabeled monomers, which are immediately trapped by excess biotinylated monomers, is the rate-limiting step. In a typical exchange experiment 37–45% of total label was bound after long incubation times (Fig. 2).

The reason for the ≈40% endpoint in the binding curve is shown in Fig. 3. The extent of biotin modification of CAP was assayed by incubating biotinylated protein with excess affinity resin, to which >80% of these dimers bind specifically. Addition of SDS, which results in the release of nonbiotinylated monomers (Fig. 1*b*), reduces the binding to <50%. Since SDS does not affect the biotin–streptavidin interaction (ref. 14 and BRL streptavidin-agarose product insert), we conclude than 80% of the dimers, but less than half of the monomers, are biotinylated. Thus, even if complete exchange of radiolabeled and biotinylated proteins is achieved, less than half of the radioactive monomers will recombine with biotinylated monomers and subsequently be bound to the resin.

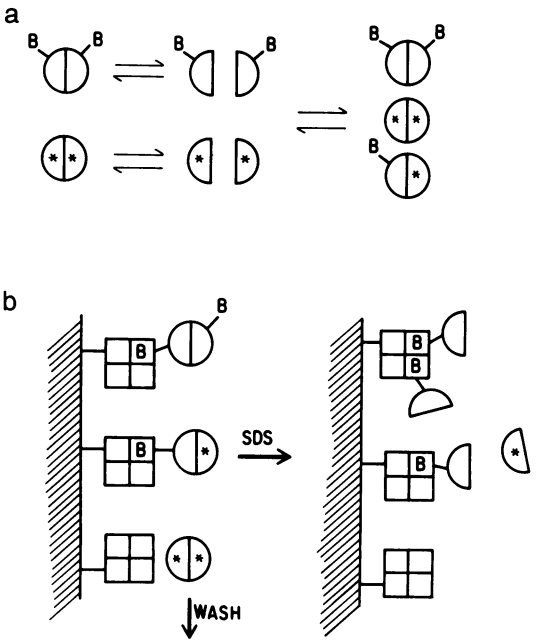

FIG. 1. Schematic depiction of subunit exchange assay. Full circles and half circles represent dimers and monomers, respectively. B indicates biotin label; asterisk indicates incorporated radiolabel. Divided squares represent streptavidin tetramers that are attached to an agarose matrix. (*a*) Exchange reaction. (*b*) Binding of exchange mixture to affinity resin.

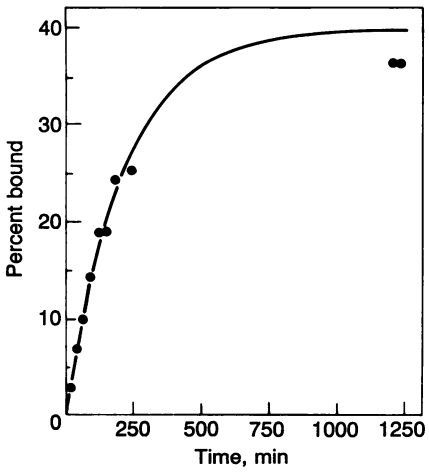

FIG. 2. Subunit exchange kinetics in absence of cAMP. CAP concentration was 3.7×10^{-8} M in storage buffer. Solid line is least-squares fit of a typical experiment to a single exponential assuming a 40% endpoint, where $k_{ex} = 4.7 \times 10^{-3}$ min^{-1}. Kinetic experiments were done at 37°C in PE buffer (20 mM potassium phosphate, pH 7/1 mM EDTA/3 mM dithiothreitol/0.05% Tween 80/8% bovine serum albumin) except as indicated. Biotinyl-CAP and [^{35}S]CAP were mixed in PE in a molar ratio of 9:1 and diluted to the concentration indicated. At various times 100-μl aliquots were removed and assayed for subunit exchange.

In general the exchange kinetics fit a single exponential decay at early times ($t < \tau$). With an endpoint of 40%, the exchange rate constant $k_{ex} = (3.4 \pm 0.7) \times 10^{-3}$ min^{-1} (τ = 300 min) in the absence of cAMP and DNA. This rate changes < 2-fold between 10^{-8} M and 2×10^{-6} M CAP. We also observe that k_{ex} is essentially independent of ionic strength from 0 to 0.5 M KCl at pH 7 and largely insensitive to pH

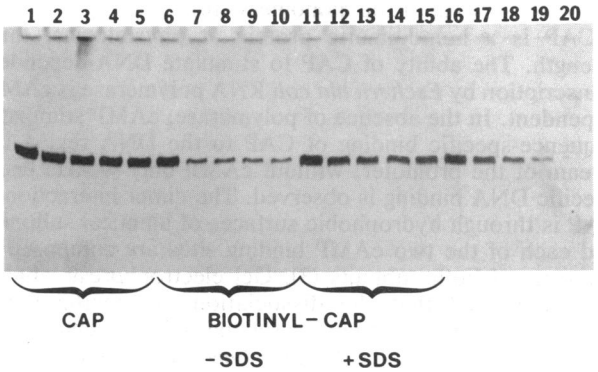

FIG. 3. Assay of CAP biotinylation by absorption onto streptavidin-agarose. After incubation with resin in binding buffer, aliquots of supernatant were mixed with 0.1% SDS sample buffer, heated, and resolved by SDS/15% polyacrylamide gel electrophoresis. Lanes: 1–5, unreacted control; 6–10, biotinyl-CAP; 11–15, biotinyl-CAP with 0.1% SDS added to the resin incubation mixture. Each assay contained 1.4 μg of CAP. Each set from left to right contained 0, 10, 20, 30, or 40 μl of streptavidin-agarose in 140 μl of binding buffer. Lanes 16–20, 1-, 2-, 4-, 8-, and 16-fold dilutions of sample 6, respectively.

between pH 6 and 8. It should be emphasized that the exchange rate measured in this assay, k_{ex}, is k_{diss} of the radiolabeled CAP ([^{35}S]CAP), since dissociation of the ^{35}S-labeled protein is the rate-determining step. This is because biotinyl-CAP is in excess and the rate of association is much faster than dissociation under our conditions.

Detection of Dissociated CAP Monomers. The exchange assay described above indirectly detects transient monomers of [^{35}S]CAP. This general strategy of detecting monomers by subsequent affinity binding of radiolabeled heterodimers can be adapted to quantitate the fraction of monomers in equilibrium with dimer. At suitably low protein concentrations a significant fraction of the available CAP will be in monomeric form. If the concentration of the CAP in the system is suddenly raised, the fraction of monomer will drop due to mass action. This sequence of monomerization followed by trapping of free monomers is accomplished experimentally in the following manner: [^{35}S]CAP is diluted in standard buffer and allowed to equilibrate. A small volume of highly concentrated biotinyl-CAP is then added, raising the total concentration of CAP several hundred- to several thousandfold. The free radiolabeled monomers rapidly recombine with biotinylated monomers, which are in excess, and the mixture is immediately loaded on the streptavidin separation column to prevent further exchange. The fraction of radiolabel retained by the affinity resin corresponds to the fraction of monomers that were free at low concentration. Results of such an experiment done over a range of concentrations (Fig. 4*a*) indicate that the dimer dissociates in the subnanomolar range. Fig. 4*b* is a linearized plot based upon a simple dissociation model

$$2m \overset{K_a}{\rightleftarrows} D$$

where m is the monomer, D is the dimer, and the equilibrium expression is $[D] = K_a[m]^2$. The line is a linear fit to the first

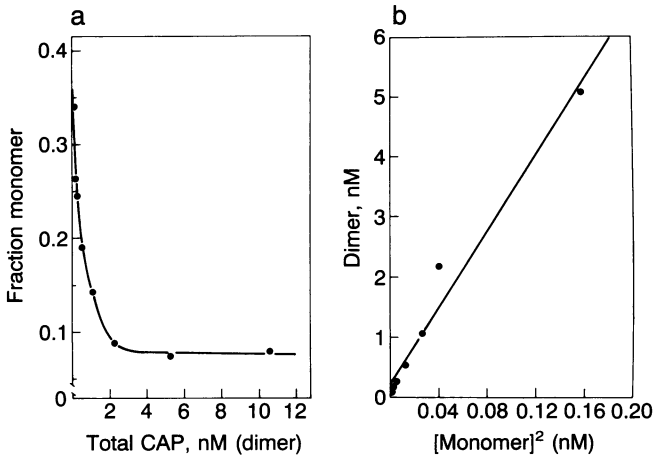

FIG. 4. Dissociation of CAP by dilution in absence of cAMP. [^{35}S]CAP was diluted to the indicated concentrations and incubated for 24 hr to reach equilibrium. Free monomers of [^{35}S]CAP were trapped by addition of excess biotinyl-CAP and, after a 1-hr incubation, were assayed for hybridization. (*a*) Plot of fraction monomer versus initial CAP concentration. (*b*) Linearized dissociation plot; slope = $K_a = (3 \pm 1) \times 10^{10}$ M^{-1}. [^{35}S]CAP was diluted to a final concentration of 0.1–10 nM in PE buffer and incubated overnight at 37°C. Biotinyl-CAP stock was added to a final concentration of 400 nM, mixtures were incubated for 1 hr, and aliquots were assayed as described for Fig. 2. Parallel samples were assayed in which a stock solution of [^{35}S]CAP was diluted to appropriate concentrations immediately before addition of biotinyl-CAP. No concentration dependence was detected in these controls, with an average 2.2% of total label bound. Both plots were corrected for background. (Fraction monomer = % bound/40%.)

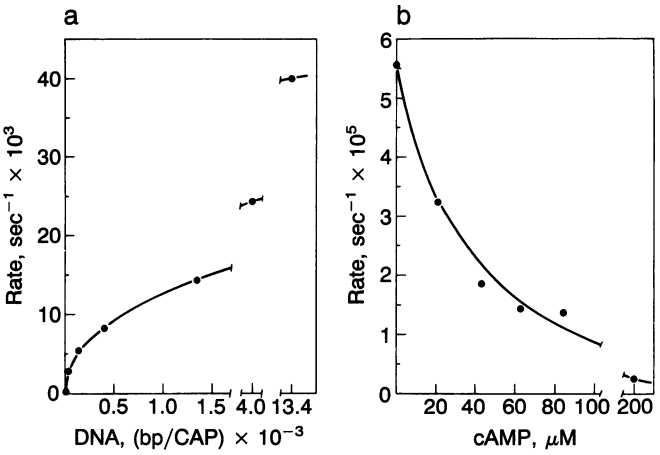

FIG. 5. Influence of ligands on CAP subunit exchange. (*a*) Acceleration of CAP exchange by calf thymus DNA. Measurements were made in PE buffer by using a high-salt quench protocol. A stock mixture of biotinyl-CAP and [^{35}S]CAP was added to PE buffer containing 0–1.3 × 10^{-4} M [concentration of base pairs (bp)] sonicated, deproteinated calf thymus DNA, with average length 400 bp. The final CAP concentration was 10.4 nM. The exchange reaction was quenched by adding 100 μl of reaction mixture to 20 μl of 5 mM cAMP/2.5 M NaCl and mixing. After at least 5 min streptavidin-agarose was added and assayed as above. Control experiments indicated <5% sample drift several hours after quenching. (*b*) Inhibition of CAP monomerization by cAMP. Exchange kinetics of CAP protein were assayed in PE buffer by the standard assay protocol (Fig. 2). The final concentration of CAP was 89 nM, the concentration of cAMP was varied from 0 to 200 μM.

seven experimental points in Fig. 4*a* with $K_a = (3 \pm 1) \times 10^{10}$ M^{-1}.

Exchange Kinetics in the Presence of Bulk DNA and cAMP. The effect of DNA on subunit exchange was assessed by mixing CAP and DNA at low ionic strength. After various incubation intervals, DNA binding was abolished and CAP dimers were stabilized by addition of NaCl and cAMP to aliquots of the CAP/DNA mixture. As shown in Fig. 5*a*, added nonspecific DNA dramatically accelerates CAP subunit exchange. At a ratio of DNA base pairs (bp) to CAP of 135 the apparent exchange rate is accelerated ≈100-fold; thereafter the exchange rate rises more gradually. The dimer lifetime is reduced to less than 1 min when the bp/CAP ratio exceeds 13,000, corresponding to an ≈1000-fold acceleration in the rate of subunit exchange. Acceleration is also observed at physiological ionic strength, but higher protein and DNA concentrations are required (data not shown) in order to compensate for the weaker binding due the ionic dependence of the DNA binding reaction (15).

cAMP is known to bind and cause conformational changes in CAP dimer in the absence of DNA (16, 17). The cyclic nucleotide is also the natural effector molecule that stimulates sequence-specific binding to DNA (15, 18, 19) and enhances nonspecific binding (15). Therefore, we wished to investigate what effect cAMP has upon the stability of CAP dimer. Fig. 5*b* shows that the consequence of cAMP binding is to slow CAP subunit exchange. This result is consistent with crystallographic work showing that the cAMP binding sites are located at the interface between the two subunits (1, 3).

DISCUSSION

Subunit Assay. We have presented a method for detecting protein subunit exchange that is analogous to isotope exchange experiments. Binding of radiolabel to the biotin affinity resin is a consequence of subunit exchange, presum-

ably through a monomer intermediate. The monomers may be transient, as in the initial exchange experiment, or in equilibrium with dimer, as in the experiments reported in Fig. 4. The high stability of CAP dimers when at submillimolar concentrations while dissociated from DNA has allowed us to monitor the rapid exchange kinetics of CAP bound to DNA by a mix-and-quench procedure.

The method described herein is general, insofar as label can be introduced into a protein of interest without affecting activity. Weber and colleagues (20–24) and others (25) have studied the dissociation of various oligomeric proteins by high pressure and subsequent reassembly at atmospheric pressure. The association constants reported for dimeric proteins are similar in magnitude to the value we report for CAP. Their approach relies on perturbation of equilibrium conditions and reaches nanomolar concentrations of protein with fluorescence detection (24). The sensitivity of our assay is apparent from the conditions we report, with concentrations of 35 nM typical and 10 nM achievable in kinetic experiments. Utilizing protein that has been labeled to a modest specific activity (typically 10–15 Ci/mmol of CAP dimer) we have measured the equilibrium extent of protein dissociation in the 10 pM concentration range.

Levitzki and Tenenbaum (26, 27) studied the reassembly steps of lactate dehydrogenase tetramer by "hybridization" of heart and muscle isoenzyme subunits. Although their assay is conceptually related to the one described in this article, it requires isoenzymes that can be resolved electrophoretically. Antonini *et al.* (28) reported a chromatographic retardation method for detecting subunit exchange between multimeric proteins covalently bound to a solid matrix and protein in solution. In our method the exchange reaction proceeds completely under solution conditions, allowing greater control over solution composition as well as much better time resolutions and sensitivity.

Ligand Effects. The inhibitory effect of cAMP on CAP subunit exchange can most simply be explained as indicating that cAMP binds only to the dimer and must dissociate before monomerization can occur. Similar slowing of subunit exchange due to cAMP is apparent in the presence of added nonspecific DNA, although lower concentrations of cAMP are required to achieve the same effect (data not shown). This is consistent with the observation that cAMP enhances the affinity of CAP for nonspecific DNA (15); if cAMP binding strengthens DNA affinity, then thermodynamic considerations (29) require that DNA will equally enhance cAMP affinity. We have observed a similar pattern of acceleration and inhibition of exchange with DNA containing the strong CAP binding site from the *lac* operon in the absence and presence of cAMP (unpublished data).

The acceleration of subunit exchange by DNA is the more surprising observation, since in this instance ligand (DNA) binding results in a *reduction* of dimer kinetic stability despite the interaction of DNA with both subunits (3, 18, 30). Apparently, a more favorable interaction of monomer with DNA stabilizes the monomer form compared with dimer. Pressure-dissociation experiments with yeast hexokinase also have indicated that noncompetitive ligands can have opposite effects on dimer stability (23).

The antagonistic effects of cAMP and DNA on CAP dimer stability suggest consideration of an alternative model for the regulation of gene activation by CAP: cAMP may act as an effector of specific binding by modulating the monomer–dimer equilibrium of CAP. It has long been recognized that cAMP stimulates sequence-specific binding by CAP (10, 17, 30, 31). However, the functional model based upon biochemical (16, 32) and structural data (3) is that cAMP alters protein conformation, specifically the orientation of DNA-binding domains, in a manner similar to the effect of trytophan binding on *trp* repressor (33). Our proposal is that a major influence of cAMP on CAP is to increase the cooperativity between subunits with respect to DNA binding: cAMP-induced dimerization greatly increases DNA binding affinity by forming a single molecule from two subunits.

Variation of dimer stability has been shown to be important in regulatory mechanisms. Cleavage of bacteriophage λ repressor protein by RecA protein reduces repressor affinity for operator by 3 orders of magnitude. This is due to the loss of dimer interaction between the amino-terminal DNA-binding domains (7). We will present evidence in a forthcoming paper that nonspecific DNA induces stable monomerization of CAP. Thus, binding of cAMP may alter the affinity of CAP for the promoter and hence transcriptional activation by enhancing dimer stability. Recent experimental evidence indicates that estrogen binding plays a role in the formation of stable human estrogen receptor dimers that are active in gene regulation (34). All of these examples are the result of the altered thermodynamics of the dimer interaction.

The theme of mixing different combinations of regulatory protein subunits to form mixed hybrids with differing sequence-binding properties has been suggested in several eukaryotic systems (8, 9, 35–39). Our kinetic findings introduce a dynamical aspect to this model: exchange of subunits bound to DNA allows rapid reprogramming of protein specificity. For example, the regulatory effect of a homodimer A_2 can be cancelled by synthesis of B subunits, resulting in disproportionation to AB, without delay for protein turnover to remove A_2. Disproportionation of this type could also be important in three-dimensional pattern formation. If two dimeric regulatory proteins A_2 and B_2 migrate to, or are transported into, a boundary region, they could rapidly rearrange to AB dimers, resulting in a spatially defined transition in gene activity between the states characteristic of A_2 and B_2. If the heterodimer AB is much more stable than the homodimers A_2 and B_2, or if AB binds DNA more tightly than A_2 or B_2 (38), then the topographical transition can be very sharp, since the only homodimer present will be that whose subunits are in local stoichiometric excess.

We thank Nanibushan Dattagupta for his helpful suggestions and discussions. We gratefully acknowledge Tarmo Ruusala for his tireless debates throughout the progress of this work and for his critical reading of the manuscript. This work was supported by Grant GM34205 from the National Institutes of Health.

1. McKay, D. B. & Steitz, T. A. (1981) *Nature (London)* **290,** 744–749.
2. Pabo, C. O. & Sauer, R. T. (1984) *Annu. Rev. Biochem.* **53,** 293–321.
3. McKay, D. B., Weber, I. T. & Steitz, T. A. (1982) *J. Biol. Chem.* **257,** 9518–9524.
4. Otwinowski, Z., Schevitz, R. W., Zhang, R.-G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F. & Sigler, P. B. (1988) *Nature (London)* **335,** 321–329.
5. Ohlendorf, D. H., Anderson, W. F., Lewis, M., Pabo, C. O. & Matthews, B. W. (1983) *J. Mol. Biol.* **169,** 757–769.
6. Wolberger, C., Dong, Y., Ptashne, M. & Harrison, S. C. (1988) *Nature (London)* **335,** 789–795.
7. Sauer, R. T., Pabo, C. O., Meyer, B. J., Ptashne, M. & Backman, K. C. (1979) *Nature (London)* **279,** 396–400.
8. Miller, A. M., MacKay, V. L. & Nasmyth, K. A. (1985) *Nature (London)* **314,** 598–603.
9. Laughton, A., Carroll, S. B., Storfer, F. A., Riley, P. D. & Scott, M. P. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 253–262.
10. Fried, M. G. & Crothers, D. M. (1984) *J. Mol. Biol.* **172,** 263–282.
11. Ghosaini, L, Brown, A. M. & Sturtevant, J. M. (1988) *Biochemistry* **27,** 5257–5261.
12. Wray, W., Boulikas, T., Wray, V. P. & Hankock, R. (1981) *Anal. Biochem.* **118,** 197–203.
13. Brown, A. (1987) Thesis (Yale University, New Haven, CT).
14. Ross, S. E., Carson, S. D. & Fink, L. M. (1986) *Biotechniques*

4, 350–354.
15. Takahashi, M., Blazy, B., Baudras, A. & Hillen, W. (1983) *J. Mol. Biol.* **167,** 895–899.
16. Eilen, E. & Krakow, J. S. (1977) *J. Mol. Biol.* **114,** 47–60.
17. Wu, F. Y.-H., Nath, K., & Wu, C.-W. (1974) *Biochemistry* **13,** 2567–2572.
18. de Crombrugghe, B., Busby, S. & Buch, H. (1984) *Science* **224,** 831–838.
19. Fried, M. G. & Crothers, D. M. (1984) *J. Mol. Biol.* **172,** 241–262.
20. King, L. & Weber, G. (1986) *Biochemistry* **25,** 3632–3637.
21. King, L. & Weber, G. (1986) *Biochemistry* **25,** 3637–3640.
22. Weber, G. & Drickamer, H. G. (1983) *Q. Rev. Biophys.* **16,** 89–112.
23. Ruan, K. & Weber, G. (1988) *Biochemistry* **27,** 3295–3301.
24. Ruan, K. & Weber, G. (1989) *Biochemistry* **28,** 2144–2153.
25. Seifert, T., Bartholmes, P. & Jaenicke, R. (1985) *Biochemistry* **24,** 339–345.
26. Levitzki, A. & Tenebaum, H. (1974) *Isr. J. Chem.* **12,** 327–337.
27. Tenenbaum-Bayer, H. & Levitzki, A. (1976) *Biochim. Biophys. Acta* **445,** 261–279.
28. Antonini, E., Fanelli, M. R. R., Chiancone, E. (1975) in *Protein Ligand Interactions*, eds. Sund, H. & Blauer, G. (de Gruyter, Berlin), pp. 45–59.
29. Weber, G. (1975) *Adv. Protein Chem.* **29,** 241–262.
30. Smitz, A. (1981) *Nucleic Acids Res.* **9,** 277–292.
31. Majors, J. (1975) *Nature (London)* **256,** 672–673.
32. Ebright, R. H., Le Grice, S. F. J., Miller, J. P. & Krakow, J. S. (1985) *J. Mol. Biol.* **182,** 91–107.
33. Zhang, R.-G., Joachimiak, A., Lawson, C. L., Schevitz, R. W., Otwinowski, Z. & Sigler, P. B. (1987) *Nature (London)* **327,** 591–597.
34. Kumar, V. & Chambon, P. (1988) *Cell* **55,** 145–156.
35. Shepard, J. C. W., McGinnis, W., Carrasco, A. E., DeRoberts, E. M. & Gehring, W. J. (1984) *Nature (London)* **310,** 70–71.
36. Oleson, J., Hahn, S. & Guarente, L. (1987) *Cell* **51,** 953–961.
37. Chodosh, L. A., Baldwin, A. S., Carthew, R. W. & Sharp, P. A. (1988) *Cell* **53,** 11–24.
38. Chodosh, L. A., Oleson, J., Hahn, S., Baldwin, A. S., Guarente, L. & Sharp, P. A. (1988) *Cell* **53,** 25–35.
39. Kouzarides, T. & Ziff, E. (1988) *Nature (London)* **336,** 646–651.