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DNA looping between the origin of replication of Epstein–Barr virus and its enhancer site: Stabilization of an origin complex with Epstein–Barr nuclear antigen 1

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ABSTRACT **Epstein–Barr nuclear antigen 1 (EBNA-1) is the only viral protein required to support replication of Epstein–Barr virus during the latent phase of its life cycle. The DNA segment required for latent replication, *oriP*, contains two essential binding regions for EBNA-1, termed FR and DS, that are separated by 1 kilobase pair. The FR site appears to function as a replicational enhancer providing for the start of replication at the DS site. We have used electron microscopy to visualize the interaction of EBNA-1 with its binding sites and to study the mechanism for communication between the FR and DS sites. We have found that DNA-bound EBNA-1 forms a DNA loop between the FR and DS sites. From these results, we suggest that EBNA-1 bound to the replicational enhancer acts by a DNA-looping mechanism to facilitate the initiation of DNA replication. Occupancy of the DS site alone is highly sensitive to competition with nonspecific DNA. In contrast, occupancy of the DS site by looping from FR is largely resistant to the competitor DNA. These experiments support the concept that enhancers act in cis from nearby sites to provide a high local concentration of regulatory proteins at their target sites and to stabilize regulatory interactions.**

Epstein–Barr virus (EBV) can establish a latent state in which the EBV genome is maintained as a circular plasmid (1, 2). Studies with plasmids derived from EBV DNA have established the functional requirements for replication during the latent state. The Epstein–Barr nuclear antigen 1 (EBNA-1) is the only viral protein required for replication from the EBV latent origin of replication, *oriP* (3, 4). The *oriP* region is composed of two essential segments separated by about 1 kilobase pair (kbp): (*i*) a family of repeats (FR) with 20 tandem copies of a 30-bp sequence and (*ii*) a dyad symmetry region (DS) with 4 copies of the same repeat (5, 6). EBNA-1 protein binds specifically to the FR and DS sequences (7–10). Since EBNA-1 is the only viral protein required for *oriP* function, EBNA-1 binding to the FR and DS sequences is probably the initial step in directing the cellular replication machinery to the viral origin. The DS region has been defined as the initiation site for DNA replication (11, 12). Thus the FR region appears to function as a replicational enhancer. The FR region can also act as a transcriptional enhancer (13).

An understanding of the initiation of EBV replication requires a knowledge of the mechanism of communication between the replicational enhancer FR and the initiation site for replication 1 kbp away at DS. A large number of experiments on controlled replication, recombination, and transcription of DNA in prokaryotes and eukaryotes have led to the unifying principle that DNA-bound proteins typically interact to generate a multiprotein regulatory complex in which the intervening DNA is looped or wound (14). For transcriptional regulation, recent work has demonstrated association of DNA-bound proteins between sites involved in enhancer function by the bacterial NtrC protein (15), the mammalian Sp1 protein (16, 17), the viral bovine papilloma E2 protein (18), and the Sp1 and E2 proteins (19). Our work on EBNA-1 has been directed toward two questions: Does replicational enhancement involve the protein–protein association of DNA-bound EBNA-1? Why do enhancers typically act only from relatively close sites on the same DNA molecule?

In the work reported here, we have used electron microscopy to study the interaction of EBNA-1 with its specific binding sites and to examine the ability of DNA-bound EBNA-1 to carry out protein–protein interactions. Our results demonstrate that EBNA-1 bound at the FR and DS sites associates to loop the intervening DNA. As reported in the accompanying paper, Frappier and O'Donnell (20) have also used electron microscopy to show this looping interaction. Based on the sensitivity of the EBNA-1 interaction at the DS site to competition with nonspecific DNA, we suggest that EBNA-1 acts in cis from the FR enhancer to stabilize the interaction of the protein at the DS site.

MATERIALS AND METHODS

DNA and Protein. The DNA fragments were isolated from the plasmid p972 (39). The FR fragment was the *Mlu* I–*Hin*dIII fragment from p972. The DS fragment was the *Sca* I–*Eco*RV fragment from p972. The FR–DS fragment was the *Eco*RI–*Hin*dIII fragment from p972. The purified fragments were isolated after electrophoresis in a 1.5% agarose gel. The purification of EBNA-1 protein is described in detail elsewhere (39). In brief, EBNA-1 was overproduced from a simian virus 40 vector in CV-1 cells and purified by chromatography on a heparin-agarose column and a DNA affinity column containing the binding sequence for the protein. The EBNA-1 was shown to be biologically active by transfection into mammalian cells along with a reporter plasmid containing the *oriP* enhancer element, a herpes simplex thymidine kinase promoter, and the gene for luciferase (39).

Electron Microscopy. The reaction mixtures contained 0.25 nM DNA fragment, 5–20 nM EBNA-1, 25 mM Tris Cl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM dithiothreitol, and 10% (vol/vol) glycerol. In some experiments, 870 nM 35-mer duplex DNA competitor was added (20-fold excess in terms of base pairs). The DNA sequence of the competitor (top strand) was 5′-GAGCTTCTCGAGCTG-CACGACGTTGTAAAACGACG-3′. The EBNA-1 protein was preincubated in the reaction buffer for 10 min at 30°C. The mixture was cooled to room temperature, and the DNA was added. If present, competitor DNA was added first, followed 30 sec later by the DNA with the specific binding

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Abbreviations: EBV, Epstein–Barr virus; EBNA-1, Epstein–Barr nuclear antigen 1.

sites. The reaction mixture was incubated at room temperature for 2 hr. The sample was loaded on grids directly without dilution or fixation. Electron microscopy was carried out by the polylysine technique (21, 22). Grids were rotary shadowed with tungsten. Magnification was approximately ×30,000.

RESULTS

Interaction of EBNA-1 with FR and DS Target Sites. EBNA-1 binds specifically to the 30-bp repeat sequences that define the FR and DS binding sites, as judged by DNase I protection, gel retardation, and filter-binding assays (7–10). We have used electron microscopy to study the interaction of EBNA-1 with its binding sites because of the special capacity of this technique to visualize the interaction between DNA-bound proteins (14, 22). We first analyzed the interaction between EBNA-1 and the separated FR and DS sites carried on DNA restriction fragments (Fig. 1).

Examples of EBNA-1 protein bound at the FR site are shown in Fig. 2; quantitative data are presented in Table 1. We observed two types of DNA–protein complexes: "simple complexes" involving a single DNA molecule (Fig. 2 *a–d*) and "multiple complexes" in which two or more DNA molecules were joined by bound EBNA-1 (Fig. 2 *e* and *f*). The simple complexes were variable in size, indicating a lack of strong cooperativity in the association reaction at the 20 tandem repeats (Fig. 2 *a–d*). The location of the DNA–protein complex was in the region expected from the position of the FR site on the restriction fragment (data not shown). The occurrence of multiple complexes indicates that EBNA-1 is capable of joining distant DNA segments by a protein–protein association reaction. The multiple complexes at the FR site were effectively eliminated by a double-stranded competitor oligonucleotide, although the simple complexes were not highly sensitive to the excess nonspecific DNA (Table 1, lines 2 and 3). We presume that the intermolecular complexes require a higher-order nucleoprotein structure at the FR site than is needed to visualize the simple complex [where one EBNA-1 dimer of molecular weight 100,000 should be detectable (e.g., refs. 16 and 22)].

Examples of EBNA-1 bound at the DS site are shown in Fig. 3; quantitative data are given in Table 2. As for the FR site, we observed simple complexes (Fig. 3*a*) and multiple complexes (Fig. 3 *b* and *c*). Because the DS site is near the end of the DNA restriction fragment (Fig. 1), the specificity of the binding reaction is obvious from inspection of Fig. 3. The formation of both simple and multiple complexes was markedly reduced by the presence of the oligonucleotide competitor (Table 2, lines 2 and 3). This observation is consistent with previous data showing that EBNA-1 binds less tightly to the DS site than to the FR site (8, 10).

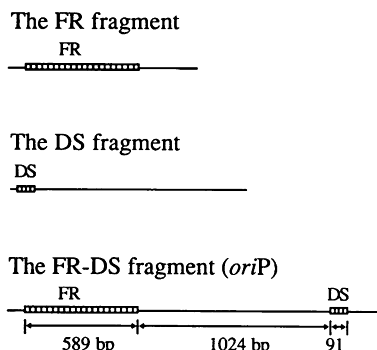

FIG. 1. The *oriP* region of EBV. The *oriP* DNA restriction fragment used in the experiments has a length of 1963 bp; the restriction fragment carrying only the DS binding site has a length of 1203 bp; and the fragment with only the FR site has a length of 987 bp. The location and extents of the FR and DS binding sites for the EBNA-1 protein are drawn to scale (striped bars).

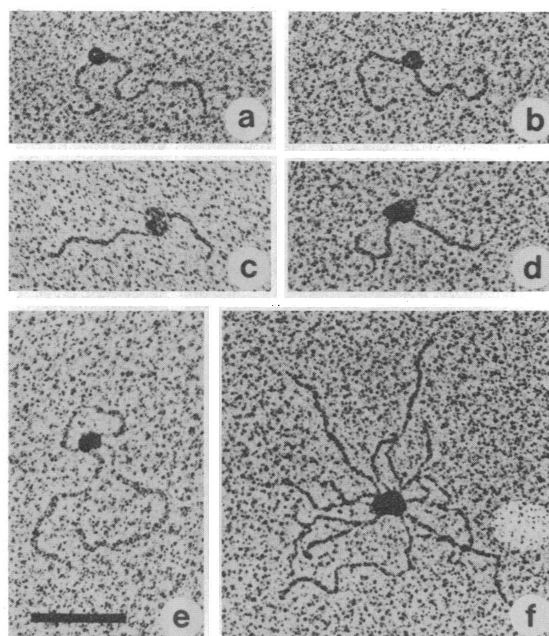

FIG. 2. EBNA-1 protein bound to the FR site. The DNA was the restriction fragment shown in Fig. 1 carrying only the FR site. The panels display the different types of DNA–protein complexes that were observed: simple complexes with EBNA-1 bound to one DNA molecule (*a–d*); double complex with two DNA molecules associated by bound EBNA-1 (*e*); and more complicated multiple complex (*f*). (Bar = 0.1 μm.)

DNA Looping Between FR and DS Sites. The major goal of our work has been to understand the mechanism by which the FR site functions as a replicational enhancer providing for the initiation of replication at the DS site. To approach this goal, we studied the interaction of EBNA-1 with the complete *oriP* region of DNA carrying both the FR and DS sites. In addition to the linear DNA–protein complexes of Fig. 2 and Fig. 3, we observed that 5% of the *oriP* molecules carried DNA loops with bound EBNA-1 at the base of the loop (Fig. 4; Table 3, line 1). The looping interaction was not observed for the DNA fragment with only DS (which was nearly as long as the *oriP* DNA) (Table 3, line 3). To provide more data on the speci-

Table 1. DNA–protein complexes formed between EBNA-1 protein and the FR segment of DNA

EBNA-1, nM	Competitor	Percent of total observed structures		
		Free DNA	Simple complex	Multiple complex
5	–	36	22	42
10	–	16	26	58
10	+	47	52	0.5
20	–	4	36	60

Examples of simple complexes and multiple complexes are shown in Fig. 2. The DNA with the FR site for EBNA-1 was the *Mlu* I–*Hin*dIII fragment from the plasmid p972 (Fig. 1). The standard reaction mixture contained 0.25 nM DNA fragment, 25 mM Tris Cl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM dithiothreitol, and 10% glycerol. For the data of line 3, 870 nM 35-mer double-stranded competitor DNA was added (20-fold excess in terms of base pairs). The reaction mixture was incubated at room temperature for 2 hr. The sample was loaded on grids directly without dilution or fixation. The total number of DNA–protein complexes counted for each reaction was 200.

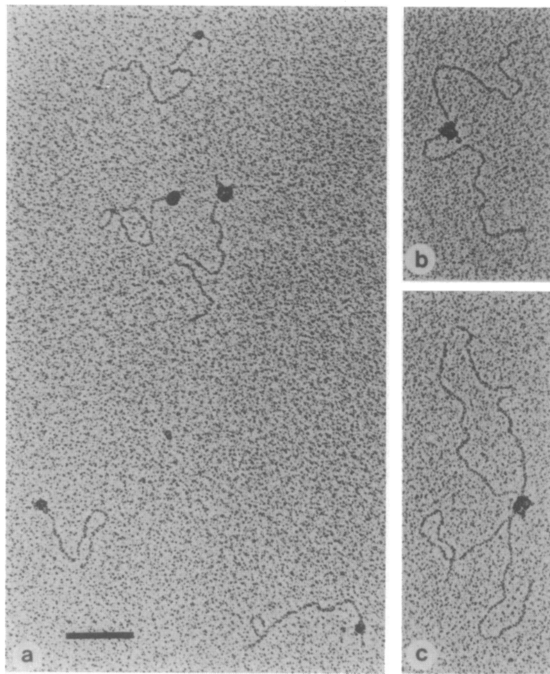

FIG. 3. EBNA-1 protein bound to the DS site. The DNA was the restriction fragment shown in Fig. 1 carrying only the DS site. The panels display the different types of DNA–protein complexes that were observed: simple complexes (*a*); double complex (*b*); and more complicated multiple complex (*c*). (Bar = 0.1 μm.)

ficity of the looping interaction, we measured the lengths of the loops and the DNA tails for looped structures like those of Fig. 4. The DNA loops fell into the size range expected for a looping interaction between the FR and DS sites (Fig. 5). The DNA tail lengths from the base of the loops to the end of the DNA were also consistent with a specific looping interaction (the distribution for FR is broad because of the long repeated binding sequence).

From the experimental results presented in Figs. 4 and 5 and Table 3, we conclude that DNA-bound EBNA-1 forms a DNA loop between the FR and DS sites. We presume that this looping interaction is the physical basis for the communication between the FR and DS sites required in the initiation of DNA replication from *oriP*. Since replication begins in the DS region, we assume that the EBNA-1 binding at DS is the critical interaction that targets the replication proteins to the viral origin of replication. A likely role of the replicational enhancer FR is to provide regulated occupancy of the DS site. A biochemical prediction of this concept is that stable association of EBNA-1 with the DS site might require the looping interaction with FR under conditions analogous

Table 2. DNA–protein complexes formed between EBNA-1 protein and the DS segment of DNA

		Percent of total observed structures		
EBNA-1, nM	Competitor	Free DNA	Simple complex	Multiple complex
5	–	22	47	31
10	–	9	28	63
10	+	93	6	1
20	–	3	28	69

Examples of simple and multiple complexes are shown in Fig. 3. The DNA with the DS site was the *Sca* I-*Eco*RV fragment from the plasmid p972 (Fig. 1). DNA-binding reactions and electron microscopy were carried out as for Table 1. The total number of DNA–protein complexes counted for each reaction was 200.

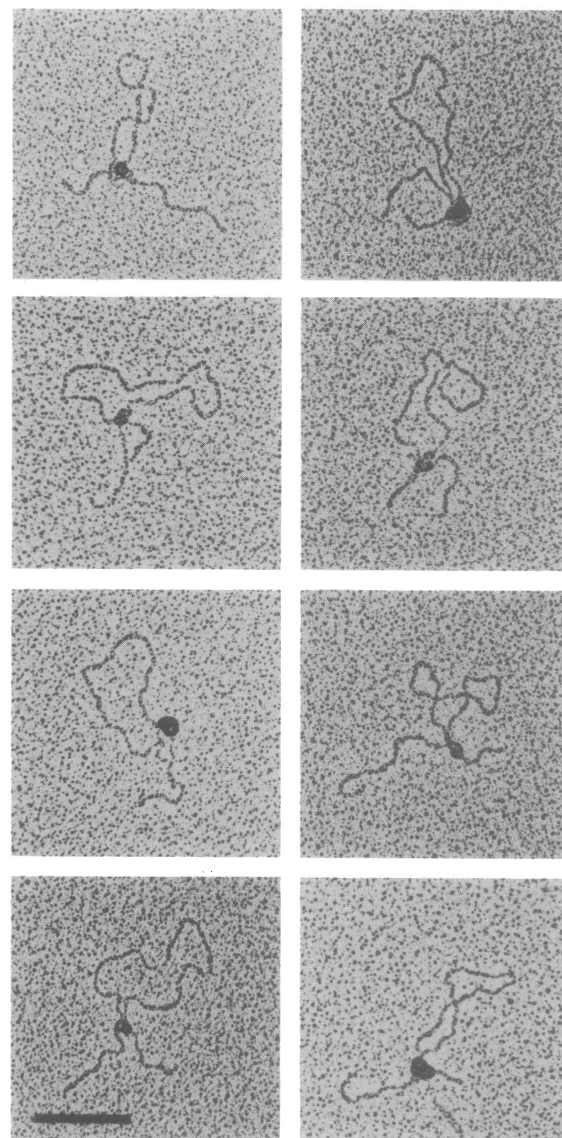

FIG. 4. DNA loops formed by association of EBNA-1 with both the DS and FR sites. The DNA was the *oriP* restriction fragment shown in Fig. 1. (Bar = 0.1 μm.)

to those found *in vivo*, in which there is a large excess of nonspecific DNA.

To test the stability of the interaction of FR and DS in the presence of competing nonspecific DNA sites, we repeated the binding experiment with the *oriP* DNA in the presence of excess competing oligonucleotide. We found that the looping

Table 3. DNA–protein complexes formed between EBNA-1 protein and the complete *oriP* region of DNA

		Percent of total observed structures		
DNA	Competitor	Free DNA	Linear complex	Loop
FR–DS fragment	–	15 ± 2	80 ± 3	5 ± 1
	+	37 ± 1	58 ± 2	4 ± 1
DS fragment	–	6 ± 2	94 ± 2	<0.3
	+	92 ± 3	8 ± 3	<0.3

Linear complexes are all DNA–protein complexes with no loops (simple complexes and multiple complexes). Examples of looped DNA molecules are shown in Fig. 3. DNA-binding reactions and electron microscopy were carried out as for Tables 1 and 2. The EBNA-1 protein concentration was 10 nM. The data were calculated from four separate counts of 100 different DNA–protein complexes.

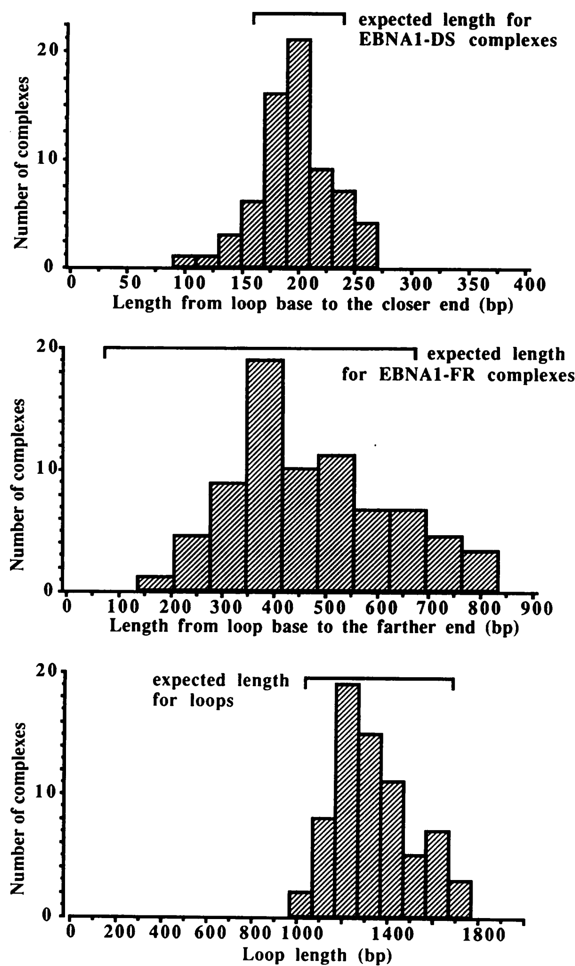

FIG. 5. Quantitative measurements from looped DNA molecules. The length of the loops and the DNA tails were measured for molecules of the type shown in Fig. 4. (*Top*) Measurements of the shorter tail segment; most of these lengths are expected to define the DS site for EBNA-1. (*Middle*) Measurements of the longer tail segment; most of these lengths are expected to define the EBNA-1 binding sites in the FR region. (*Bottom*) Measurements of the loops. Lengths were measured on a Numonics 2400 digitizing board.

interaction was not appreciably diminished by the competitor DNA (Table 3, line 2). In contrast, as noted previously, the binding of free EBNA-1 to the DS site was strongly inhibited by the competitor DNA (Table 3, line 4). We conclude that the FR site can work effectively in cis by a looping interaction to stabilize binding of EBNA-1 at the DS site. We suggest that this interaction may be the critical function of EBNA-1 acting from FR as a replicational enhancer. This point is supported by the experiments of Frappier and O'Donnell (20), in which occupancy of the DS site on *oriP* DNA was observed only by a looping interaction with FR.

The insensitivity of the cis looping interaction contrasts with the marked sensitivity of the trans interaction to the presence of competitor DNA (Table 1). Although more complicated explanations are possible, we presume that this difference is a manifestation of the principle of high local concentration. The FR and DS sites in cis and EBNA-1 constitute an interacting set that is not at free equilibrium with the competitor DNA. An EBNA-1 molecule that dissociates from the FR site will encounter the cis DS site more readily than the competitor DNA present at relatively low effective concentration (and vice versa). This effect will likely be enhanced by the "DNA-sliding" capacity of DNA-binding proteins (23). The combination of this localized concentration effect and the protein–protein association of DNA-bound EBNA-1 provide for highly efficient cis action by the enhancer.

DISCUSSION

DNA Looping and Origin Function. EBNA-1 is the only viral protein required for activation of the *oriP* origin of replication (3, 4). Thus the association of EBNA-1 with the FR and DS sequences is likely to be the initial step in activating viral DNA replication. Since the DS sequence is probably the initiation point for replication (11, 12), the binding of EBNA-1 to the DS sequence presumably directs the replication proteins of the host to start DNA synthesis at this site. The activities of EBNA-1 at the DS site would therefore be analogous to the well-defined initiator functions of *Escherichia coli* DnaA protein (24), phage λ O protein (14), and the simian virus 40 large tumor antigen (25). The critical role of the FR sequence for *oriP* appears to be that of a replicational enhancer. This assignment is consistent with the ability of FR to act as a transcriptional enhancer (13).

To investigate the communication between the FR and DS sequences, we have used electron microscopy to visualize the interaction of EBNA-1 with its binding sites. Most importantly, we have found that DNA-bound EBNA-1 forms DNA loops between the FR and DS sites. We believe that this protein–protein association of DNA-bound EBNA-1 is the basis for the communication between the enhancer sequence FR and the initiation sequence DS. The looping interaction provides for efficient occupancy of the DS site and thereby for effective assembly of the initiation complex for DNA replication.

The complex origin structure of EBV is similar to that found for the bacterial plasmids R6K (26, 27) and P1 (28). For both of these replicons, the initiator binding site at the replication start point is separated from a second, highly reiterated binding sequence for the initiator protein; the initiation and regulatory sequences can communicate by a DNA-looping interaction for both P1 (29) and R6K (30). The organization of R6K appears to be very much like that of EBV: a replicational enhancer that activates a separate initiation sequence (30). Based on evidence for complex origin structures and replicational enhancers in both eukaryotes and prokaryotes, we anticipate that a eukaryotic cellular origin of replication may involve multiple distinct regulatory sequences and thus exhibit a highly complex and extended DNA sequence.

DNA Looping and Enhancer Action. Enhancers function from distinctly separated but relatively close sites to activate a target sequence on the same DNA molecule. These features providing localized targeting are essential for biological specificity, but the physical basis of this regional interaction has been a subject of some controversy. Given the current very strong evidence that enhancer activity depends on the association of DNA-bound proteins, the most likely explanation is that enhancer sites typically anchor a regulatory protein close enough to its target protein for effective collision; that is, the enhancer provides a high local concentration of the regulator (14, 31). This concept has been supported by experiments showing that enhancer-bound transcription activators can act in trans if the enhancer and promoter are closely associated by DNA catenation or protein-mediated tethering (32–34). The highly reiterated set of EBNA-1 binding sequences in FR may serve to ensure that the requisite EBNA-1 is always available to stabilize binding to DS (subject to regulatory influences). DNA-binding proteins are expected to be especially sensitive to local concentration because of the competition provided by binding to nonspecific DNA. This sensitivity to competition with nonspecific sites may also explain why some prokaryotic regulatory proteins act preferentially in cis, at the site closest to their

location of synthesis in the coupled transcription–translation reaction in bacterial cells (35).

Our study of EBNA-1 binding to *oriP* has allowed an evaluation of the model that an enhancer provides a high local concentration of a regulator that can facilitate either DNA replication or transcription. In this study, the binding of EBNA-1 to FR and DS not only yielded a complex that looped the intervening DNA (Figs. 4 and 5) but also increased the stability of the complex of EBNA-1 with the origin of replication, DS (Table 3). We think it likely that the stabilization of the binding of EBNA-1 to DS is necessary to nucleate the formation of an initiation complex for replication of DNA. An extension of this concept is the hypothesis that EBNA-1 bound to FR enhances transcription from a nearby responsive promoter by providing a high local concentration of the regulator, and perhaps by stabilizing a key element of the transcription complex with which the regulator interacts. Based on our findings and work noted above with three distinct enhancers (32–34), we suggest that the concept of high local concentration and stabilization can explain the mechanisms of action of many enhancers.

Although probably a central feature of enhancer action, the presence of the appropriate regulatory protein at high concentration is clearly not the sole source of specificity in regulation of DNA transactions. For the highly studied transcriptional systems, the activity of regulatory proteins is often controlled posttranscriptionally, and an array of different regulatory proteins frequently determines the utilization of a promoter site (14, 36–38). For complex regulatory decisions in eukaryotes, an ensemble of regulatory sites are probably positioned close enough to the multiprotein promoter complex for effective collision, but far enough away to allow DNA looping without a severe energetic cost in DNA bending. A summation of functional activation domains (positive or negative) at the promoter might be the basis of the regulatory switch. Relatively little is known about regulation of eukaryotic origins of replication. Because EBV plasmid DNA undergoes controlled intracellular DNA replication, the study of this viral system promises to provide insights into the complex regulatory inputs that control cellular origins of replication.

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1. Nonoyama, M. & Pagano, J. S. (1972) *Nature New Biol.* **238**, 169–171.
2. Lindahl, T., Adams, A., Bjursell, G., Bornkamm, G. W., Kaschaka-Dierich, C. & Jehn, U. (1976) *J. Mol. Biol.* **102**, 511–530.
3. Yates, J. L., Warren, N. & Sugden, B. (1985) *Nature (London)* **313**, 812–815.
4. Lupton, S. & Levine, A. J. (1985) *Mol. Cell. Biol.* **5**, 2533–2542.
5. Yates, J. L., Warren, N., Reisman, D. & Sugden, B. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3806–3810.
6. Reisman, D., Yates, J. & Sugden, B. (1985) *Mol. Cell. Biol.* **5**, 1822–1832.
7. Rawlins, D. R., Milman, G., Hayward, S. D. & Hayward, G. S. (1985) *Cell* **42**, 859–868.
8. Jones, C. H., Hayward, S. D. & Rawlins, D. R. (1989) *J. Virol.* **63**, 101–110.
9. Ambinder, R. F., Shah, W. A., Rawlins, D. R., Hayward, G. S. & Hayward, S. D. (1990) *J. Virol.* **64**, 2369–2379.
10. Frappier, L. & O'Donnell, M. (1991) *J. Biol. Chem.* **266**, 7819–7826.
11. Gahn, T. A. & Schildkraut, C. L. (1989) *Cell* **58**, 527–535.
12. Wysokenski, D. A. & Yates, J. L. (1989) *J. Virol.* **63**, 2657–2666.
13. Reisman, D. & Sugden, B. (1986) *Mol. Cell. Biol.* **6**, 3838–3846.
14. Echols, H. (1990) *J. Biol. Chem.* **265**, 14697–14700.
15. Su, W., Porter, S., Kustu, S. & Echols, H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5504–5508.
16. Su, W., Jackson, S., Tjian, R. & Echols, H. (1991) *Genes Dev.* **5**, 820–826.
17. Mastrangelo, I. A., Courey, A. J., Wall, J. S., Jackson, S. P. & Hough, P. V. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5670–5674.
18. Knight, J., Li, R. & Botchan, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3204–3208.
19. Li, R., Knight, J. D., Jackson, S. P., Tjian, R. & Botchan, M. R. (1991) *Cell* **65**, 493–505.
20. Frappier, L. & O'Donnell, M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10875–10879.
21. Williams, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2311–2315.
22. Dodson, M. & Echols, H. (1991) *Methods Enzymol.*, in press.
23. von Hippel, P. H. & Berg, O. G. (1989) *J. Biol. Chem.* **264**, 675–678.
24. Kornberg, A. (1988) *J. Biol. Chem.* **263**, 1–4.
25. Kelly, T. (1988) *J. Biol. Chem.* **263**, 17889–17892.
26. Germino, J. & Bastia, D. (1983) *Cell* **34**, 125–134.
27. Filutowicz, M., Uhlenhopp, E. & Helinski, D. R. (1986) *J. Mol. Biol.* **187**, 225–239.
28. Abeles, A. L., Snyder, K. M. & Chattoraj, D. K. (1984) *J. Mol. Biol.* **173**, 307–324.
29. Chattoraj, D. K., Mason, R. J. & Wickner, S. (1988) *Cell* **52**, 551–557.
30. Mukherjee, S., Ericksen, H. & Bastia, D. (1988) *Cell* **52**, 375–383.
31. Müller, H. P. & Schaffner, W. (1990) *Trends Genet.* **6**, 300–304.
32. Müller, H.-P., Sogo, J. M. & Schaffner, W. (1989) *Cell* **58**, 767–777.
33. Dunaway, M. & Dröge, P. (1989) *Nature (London)* **341**, 657–659.
34. Wedel, A., Weiss, D., Popham, D., Dröge, P. & Kustu, S. (1990) *Science* **248**, 486–490.
35. Echols, H., Court, D. & Green, L. (1976) *Genetics* **83**, 5–10.
36. Adhya, S. (1989) *Annu. Rev. Genet.* **23**, 227–250.
37. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–378.
38. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58**, 799–839.
39. Middleton, T. & Sugden, B. (1992) *J. Virol.* **66**, in press.