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THE CONTROL OF T4 GENE 32 HELIX-DESTABILIZING PROTEIN
ACTIVITY IN A DNA REPLICATION COMPLEX¹

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ABSTRACT. 32*I protein is produced by the chymotryptic cleavage of the A domain (≈ 60 amino acid residues) from the carboxyl terminus of T4 gene 32 protein. The amino acid sequence of the A domain, and the effects of its removal on the physicochemical properties of 32 protein are reported. We have substituted 32*I protein for the intact protein in a variety of *in vitro* DNA replication reactions to probe the roles of the A domain in DNA replication.

INTRODUCTION

The bacteriophage T4 gene 32 protein may serve two essential roles in DNA replication: a functional role in promoting duplex DNA disruption required to generate the appropriate template for the DNA polymerase and a structural role in which the 32 protein-coated single-stranded DNA serves as the foundation for the assembly of the replication complex. It has been suggested that 32 protein has two protruding regions—domain A at the carboxyl terminus and domain B at the amino terminus—which are involved in 32 protein interactions with itself and with other replication proteins (1-3). These interactions may modify the DNA binding properties of 32 protein and thus control the overall activities of the replication complex. We show here that the removal of the A domain alters the DNA replication complex such that some activities are maintained while others are lost. These results, complemented by physical studies of 32*I protein, suggest that an intact A domain is essential for the control of the helix-destabilization potency of 32 protein.

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The Sequences of the 32 Protein Terminal Domains (3-6).

The amino acid sequences of the A and B regions and the specific protease cleavage sites within these regions are shown in Fig. 1. The A domain is extremely acidic; it has 14 net negative charges of which 11 cluster within the distal 29 carboxyl terminal residues (6). The removal of the A region produces a 29,000 dalton fragment designated as 32*I protein. Molecules which lack the B region (a 21 residue, basic domain) are termed 32*II protein, while those with both termini removed are termed 32*III protein.

The susceptibility of each domain to proteolysis changes when 32 protein binds to DNA: the B region is protected from proteolytic attack (2,3) while the rate of cleavage at the junction between the core and the A domain is enhanced (3). DNA binding also reveals a new Staphylococcal protease site at this junction (6). These results indicate that binding of 32 protein to DNA induces a conformational or positional change in the A domain and suggests that A peptide is connected to the core by a flexible hinge. The work of von Hippel and coworkers in this issue (7) also suggests that 32 protein undergoes a conformational change upon DNA (or polynucleotide) binding. (See discussion and Fig. 3.)

FIGURE 1. Amino acid sequences and protease cleavage sites of 32 protein A and B regions. T (trypsin), C (chymotrypsin), and S (staphylococcal protease) cleavage sites for free 32 protein are indicated. The {S} site is accessible only on DNA bound 32 protein. The B sequence is a summary of Ref. 3-5. The A sequence and its cleavage sites are from Ref. 6.

TABLE 1
PROPERTIES OF 32 PROTEIN AND ITS LIMITED PROTEOLYSIS PRODUCTS^a

Protein	32	32*I	32*II	32*III
Region(s) removed	none	A	B	A + B
Terminus		COOH	NH ₂	both
Total residues lost		≈60	≈20	
Self-association ^b	+	+	-	-
Affinity for { ss-DNA	Strong	Strong	Weak	Intermed.
DNA cellulose } ds-DNA	-	+	-	+
Helix-melting				
Poly[d(A-T)]	+	+		+
T4 DNA	-	+		+
Affinity for				
T4 DNA polymerase	+	-		
6l (Priming) protein	+	-		

^aData from Ref. 1, 2, 6 and 8; from Goto, Moise and Hosoda, and from Burke, Liu and Alberts (manuscripts in preparation).

^b32*II self-association was examined with the fragment made by Staphylococcal protease which removes the first nine amino terminal residues.

Physical Properties of 32 Protein and Its Proteolysis Products. As indicated in Table 1, interactions between 32 protein and DNA and between 32 protein molecules are altered upon the removal of its terminal domains. Intact 32 protein molecules self-aggregate to form large oligomers and the presence of the B domain (but not the A domain) is essential for self-association; 32*I protein molecules aggregate (8), while 32*II and 32*III molecules do not. Likewise, the B domain (but not the A domain) is also essential for cooperative DNA binding; 32*III protein does not show strong cooperative binding, while the binding of 32*I protein is as cooperative as that of the intact 32 protein (7,9,10).

The most dramatic change in DNA binding properties is the greatly increased potency of 32*I protein for denaturing ds-DNAs. All of the helix-destabilizing (H-D) proteins listed in Table 1 reduce the melting temperature of poly[d(A-T)] by binding selectively to single-stranded regions thus shifting the helix to coil thermodynamic equilibrium. However, intact 32 protein alone does not melt natural ds-DNAs, presumably due

to a kinetic block to the denaturation (11). The presence of the A domain seems to be essential for the maintenance of this kinetic block since both 32*I and 32*III proteins lower the melting temperature of ds-T4 DNA. These proteins also have an increased affinity for ds-DNA cellulose compared to intact 32 protein suggesting that they may invade duplex DNA regions more rapidly. Interestingly, those cleavage products lacking one-half of each terminal domain (produced by Staph. digestion) have the same affinity for DNA cellulose as the respective proteins lacking the entire terminal domains (6).

Also shown in Table 1 is the fact that 32 protein alone binds to 43 protein, the DNA polymerase (12), and to 61 protein, an RNA priming protein. These associations cannot be demonstrated for 32*I protein (8 and Burke, Liu and Alberts, manuscript in preparation).

32*I Protein Uncoupled Leading and Lagging Strand DNA Synthesis. The present "complete" T4 *in vitro* DNA replication system is comprised of seven highly purified T4 gene products: the helix-destabilizing protein (32 protein), the DNA polymerase (43 protein), the polymerase accessory proteins (44/62 protein complex plus 45 protein), and the RNA priming proteins (41 and 61 proteins) (13). By employing the entire set or subsets of these proteins with the appropriate DNA templates, we can study the full replication reaction (coupled leading and lagging strand synthesis) or partial reactions (*de novo* chain starts, chain elongation, etc.).

When the template is circular ds-PM-2 DNA which has been randomly nicked once per circle, DNA synthesis initiates at the nick and proceeds in a rolling circle mode to generate a newly replicated circle with a long, single-stranded tail. As shown in Fig. 2a, a minimum of five proteins (32, 43, 44/62 and 45 proteins) is required to obtain efficient synthesis (13,14). This "five-protein" complex copies the template with high fidelity and maintains the proper replication fork geometry (although no lagging strand synthesis occurs) (15). The addition of 41 and 61 proteins increases the incorporation two-fold (this effect is due to the presence of 41 protein alone which accelerates the rate of leading strand chain elongation (16)). Lagging strand synthesis requires *de novo* chain initiation on the displaced parental DNA strand. Pentaribonucleotides synthesized by 41 and 61 proteins are used as primers for Okazaki fragment synthesis by the five-protein complex (16). As shown in Fig. 2a, adding the rNTPs (7P + rG, U, C) produces the further two-fold increase in incorporation expected from concurrent lagging strand synthesis.

When 32*I protein is substituted into the replication complex, the resulting five-protein/32*I reaction is slightly more efficient than the normal reaction (Fig. 2b). In contrast

FIGURE 2. DNA synthesis on a singly nicked PM-2 DNA template (for reaction conditions, see Ref. 8.) The complete system (7P+rG, U, C) contains either 32 protein or 32*I protein (200 μ g/ml), polymerase, 44/62, 45, 41 and 61 proteins, dNTPs ($[^3\text{H}]\text{TTP}$), rNTPs and PM-2 DNA (39 nmoles/ml). The rNTPs (except ATP) were omitted in the limited seven-protein (7P) and in the five-protein (5P) reactions. The 41 and 61 proteins were also omitted in the five-protein reactions.

to the normal reaction, the addition of 41 and 61 proteins to the five protein/32*I complex has no effect on the observed incorporation even in the presence of all four rNTPs. This result suggests that the binding of 32*I protein to the displaced parental strand inhibits both the normal 41 protein dependent rate stimulation and the *de novo* synthesis of Okazaki fragments. The complete absence of lagging strand synthesis in the seven-protein/32*I reaction was confirmed by electron microscopic examination of these reaction products. Whereas the "tails" of the rolling circles replicated by the normal complete system were primarily double-stranded, those made in the presence of 32*I protein were always single-stranded (8).

32*I Protein Inhibits Synthesis by DNA Polymerase on Primed ss-DNA Templates. The T4 DNA polymerase alone can extend a base-paired 3'OH primer terminus on a ss-DNA template (12) such as *exo*-III-eroded λ DNA (8). When the DNA polymerase is supplemented with sufficient 32 protein to cover the initially single-stranded regions of the template, both the rate and final extent of synthesis increases (8) (Table II).

TABLE 2
 "LAGGING STRAND" DNA SYNTHESIS ON AN EXO-III
 TREATED λ DNA TEMPLATE^a

Proteins Present	Relative DNA Synthesis	
	Rates ^b	Extent ^c
Polymerase alone	1.00	1.00
+ 32 (50%) ^d	2.10	0.83
+ 32 (100%)	1.92	1.33
+ 32*I (50%)	0.35	0.50
+ 32*I (100%)	0.05	0.18
+ 32 (100%) + 44/62 + 45	2.83	2.00
+ 32*I (100%) + 44/62 + 45	2.72	3.30

^aData taken from Ref. 8.

^bAn average incorporation rate for the first five minutes.

^cTotal incorporation in 25 minutes at 23°C.

^dNumbers in parentheses indicate the percent of the ss-template DNA coated with H-D protein.

However, the addition of comparable concentrations of 32*I protein almost completely abolishes synthesis. Some incorporation is observed if the concentration of 32*I protein is reduced to one-half this amount. The polymerase accessory proteins are known to stimulate the normal 43/32 protein reactions (17). This effect is more apparent in the 32*I reaction — DNA synthesis is restored.

In summary, on both double-stranded and primed single-stranded templates, 32*I can replace 32 protein only when the polymerase functions with its accessory proteins at a 3'OH chain end. Without the accessory proteins, 32*I protein strongly blocks polymerase-catalyzed chain extension on both single-stranded templates as shown here and on nicked ds-DNA (data not shown). Specific protein-protein contacts are maintained within the five-protein/32*I complex since replacing 32*I protein with the host *E. coli* H-D protein results in an almost complete suppression of all DNA synthesis by the T4 *in vitro* system (8).

32*I Protein Blocks *de novo* Initiation of DNA Chains on Single-Stranded DNA Templates. The replication of a circular ss-DNA template requires a *de novo* priming event prior to chain elongation. In the T4 *in vitro* system all seven proteins are required to obtain RNA-primed DNA replication on a ss-fd DNA template (13). The product of this reaction, like that obtained with nicked ds-PM-2 DNA, is a rolling circle with a

TABLE III
INHIBITION OF REPLICATION ON AN fd DNA TEMPLATE BY 32*I PROTEIN^a

Ratio (w/w/w) ^b			Number of copies made	Inhibition ^c (%)
32	32*I	DNA		
25	0	1	6.43	0
25	6	1	7.20	0
25	13	1	5.45	15.9
25	25	1	2.02	69.0
13	25	1	1.20	82.1
0	25	1	0.12	98.2

^aData taken from Ref. 8. Incorporation of ³H-TTP at 30°C in 9 minutes by the complete system (see Fig. 2 legend).

^bA protein to DNA ratio of 10:1 covers the template.

^cPercent inhibition was calculated relative to the maximum synthesis observed in the absence of 32*I protein.

primarily double-stranded tail. As shown in Table III, multiple copies are normally synthesized by this *in vitro* system. However, when 32*I protein is substituted into the seven-protein complex, synthesis is completely blocked. The inhibition is competitively removed when both H-D proteins are present; at equal concentrations of the two proteins, 30% of the full activity is recovered. The simplest interpretation of these results is that 32 and 32*I proteins compete for DNA binding sites and that priming is inefficient on 32*I protein-coated DNA templates. In fact, sedimentation analyses indicate that the DNA-protein complex formed in the presence of equal concentrations of the two H-D proteins contains five-fold more 32*I than 32 protein (8).

The inhibition of the fd replication reaction in the presence of 32*I protein could arise from the reduction of either RNA primer synthesis or of primer utilization. To test for the former, RNA primer pentaribonucleotide synthesis was examined in a reaction requiring only 4l and 6l proteins, ss-DNA, and rNTPs. The addition of 32*I protein strongly inhibited pentaribonucleotide synthesis in contrast to 32 protein, which produced little inhibition. In fact, 32*I protein reduced primer synthesis by 70% as compared to 12% inhibition for intact 32 protein (8). This reduction is not as severe as the 98% inhibition of DNA replication observed in the coupled RNA/DNA synthesis reaction (Table III). Thus it seems likely that both steps (primer synthesis and subsequent utilization) are each perturbed by 32*I protein.

DISCUSSION

Within a replication complex, various properties of 32 protein may be required at different relative positions at the replication fork. For example, ahead of the nascent chain 32 protein promotes duplex opening, while at the end of the growing chain, 32 protein must permit other components of the complex (the RNA primase and the DNA polymerase) to make proper contact with the template without destabilizing the primer (either the RNA oligomer or the growing chain end itself). The 32*I protein inhibition of both RNA primer extension and of synthesis by DNA polymerase on preprimed templates may arise from a common mechanism — destabilization of the 3'OH chain terminus. The polymerase and RNA primase may interact with 32 protein directly to block invasion of the newly replicated duplex region, a control exerted through or requiring an intact A domain.

The role of the A domain in the control of 32 protein activities during DNA replication is diagrammatically presented in Fig. 3. According to von Hippel and his colleagues, the intrinsic affinity of 32 protein for short oligonucleotides

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FIGURE 3. Model for the A domain control of 32 protein activities during DNA replication.

(≤ 8 residues) is relatively salt insensitive whereas the stronger affinity for polynucleotides is very sensitive to changes in ionic strength. These electrostatic contacts lie between the core region of 32 protein and the DNA backbone (7). The absence of the A peptide permits the formation of two additional ion pairs between 32*I protein and a bound oligonucleotide, increasing the salt sensitivity of its binding towards that normally observed only during cooperative DNA binding. These electrostatic binding sites in the 32 protein core appear to be masked by the acidic A region in free or oligomer bound 32 protein molecules. The conformational change of the A domain which accompanies cooperative DNA binding appears to expose these sites.

In Fig. 3 the A domain is shown as a large, loosely folded mass of negative charges which can rotate back and forth on a "flexible hinge." In the free or oligonucleotide bound conformation (tame form) the A domain obscures basic binding sites on the peptide core. In the DNA bound conformation (active form) the A domain swivels up exposing these electrostatic binding sites. This "A mask" has been lost in 32*I protein. Thus 32*I protein inhibits reactions where the taming of a strong helix-destabilizing activity is needed.

The interactions of the remaining components of the replication apparatus with 32 protein are less clear. The fact that the five protein/32*I complex sustains the same (or even greater) level of synthesis as the normal complex suggests that a 32*I protein coated ss-DNA is in the appropriate template conformation and that specific protein-protein contacts are maintained.

Mutations (a point mutation, a small deletion, or a small addition) at the carboxyl terminal region of 32 protein have little effect on the T4 life cycle permitting near normal DNA replication (18). These results and experiments with specific amber mutants in gene 32 have been taken as evidence that the carboxyl terminal region of 32 protein is nonessential (18). This obvious contradiction to our conclusion from *in vitro* DNA replication experiments might be explained if our model presented in Fig. 3 is correct. If the A domain controls helix-destabilizing activity by using a mass of negative charges to block electrostatic interactions, then a small conformational or size change in the A domain could hardly eliminate its function — it has a mutation resistant sequence. When the deletion is so extensive as to include a part of core region concomitantly weakening DNA binding (as may be the case with specific amber mutants), taming by A domain is no longer necessary.

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REFERENCES

1. Moise, H., and Hosoda, J. (1976). *Nature* 259, 455-458.
2. Hosoda, J. and Moise, H. (1978). *J. Biol. Chem.* 253, 7457-7555.
3. Williams, K.R., and Konigsberg, W.H. (1978). *J. Biol. Chem.* 253, 2463-2470.
4. Tsugita, A., and Hosoda, J. (1978). *J. Mol. Biol.* 122, 255-258.
5. Krisch, H., Duvoisin, R.M., Allet, B., and Epstein, R.H. (this volume).
6. Hosoda, J., Kubota, I., Moise, H., and Tsugita, A. (manuscript submitted for publication).
7. Newport, J.W., Kowalczykowski, S.C., Lonberg, N., and von Hippel, P.H. (this volume).
8. Burke, R.L., Alberts, B.M., and Hosoda, J. (manuscript submitted for publication).
9. Williams, K.R., Sillerud, L.O., Schafer, D.E., and Konigsberg, W.H. (1979). *J. Biol. Chem.* 254, 6426-6432.
10. Spicer, E.K., Williams, K.R., and Konigsberg, W.H. (1979). *J. Biol. Chem.* 254, 6433-6436.
11. Jensen, D.E., Kelley, R.C., and von Hippel, P.H. (1976). *J. Biol. Chem.* 251, 7215-7228.
12. Huberman, J.A., Kornberg, A., and Alberts, B.M. (1971). *J. Mol. Biol.* 62, 39-52.
13. Liu, C.-C., Burke, R.L., Hibner, U., Barry, J., and Alberts, B.M. (1979). *Cold Spring Harbor Symp. Quant. Biol.* 43, 469-487.
14. Nossal, N.G., and Peterlin, B.M. (1979). *J. Biol. Chem.* 254, 6032-6037.
15. Sinha, N.K., Morris, C.J., and Alberts, B. (1980). *J. Biol. Chem.* 255, 4290-4303.
16. Alberts, B., Barry, J., Bedinger, P., Burke, R.L., Hibner, U., Liu, C.-C., and Sheridan, R. (this volume).
17. Alberts, B., Barry, J., Bittner, M., Davies, M., Hama-Inaba, H., Liu, C.-C., Mace, D., Moran, L., Morris, C.F., Piperno, J., and Sinha, N.K. (1977). In "Nucleic Acid-Protein Recognition" (Vogel, H.J., ed.), pp.31-63, Academic Press, New York.
18. Mosig, G., Luder, A., Garcia, G., Dannenberg, R., and Bock, S. (1979). *Cold Spring Harbor Symp. Quant. Biol.* 43, 501-515.