JOURNAL OF VIROLOGY, Dec. 1973, p. 1631-1632
Vol. 12, No. 6

Location of the T4 Gene 32 Protein Binding Site on Simian Virus 40 DNA

JOHN F. MORROW AND PAUL BERG
Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305

Received for publication 26 July 1973

The T4 gene 32 protein, which binds to single-stranded but not duplex DNA, forms a specifically located denaturation loop in covalently closed circular simian virus 40 (SV40) DNA. Cleavage of the SV40 DNA-gene 32 protein complex with a restriction endonuclease from *Hemophilus parainfluenzae* shows the loop center to be at 0.46 on the SV40 DNA map. This is within one of the regions of SV40 DNA cleaved preferentially by the single-strand-specific nuclease S_1.

Delius et al. (5) showed that incubation of simian virus 40 (SV40) supercoiled DNA [SV40(I) DNA] with T4 gene 32 protein (1) under defined conditions followed by fixation with glutaraldehyde yields circular structures with one small "denaturation loop" per molecule. In an earlier paper (9), we reported that cleavage of such structures with the site-specific restriction endonuclease, $EcoR_I$ (6, R. N. Yoshimori, Ph.D. thesis, Univ. of California, San Francisco, 1971) yields linear molecules with the midpoint of the denaturation loop located 0.45 SV40 fractional length from one end. Because the two ends of the $EcoR_I$-generated linear molecules are indistinguishable, it was impossible to assign a single fixed location for the T4 gene 32 protein binding site: that is, referring to the SV40 DNA map (Fig. 1) (10), the denaturation loop could have occurred at 0.45 or 0.55 SV40 map units (fractional length from the $EcoR_I$ cleavage site), or both.

To resolve this point, we have used a second site-specific restriction endonuclease, HpaII, to convert the gene 32 protein-SV40 DNA complexes to linear structures. HpaII cleaves SV40 DNA once at 0.73 on the SV40 map (Fig. 1)(12). Consequently, if the denaturation loop is located at 0.55 on the SV40 map, it will occur 0.18 SV40 fractional length from an end; alternatively, if the HpaII-generated linear molecules contain the denaturation loop 0.28 SV40 fractional length from an end, we could assign the T4 gene 32 protein binding site to 0.45 SV40 map position.

Circular SV40 DNA with bound gene 32 protein was prepared (5) and dialyzed to remove glutaraldehyde for 3 h at 4 C against 10 mM Tris, pH 7.4, 1 mM EDTA, and 10 mM NaCl. After addition of $MgCl_2$ to 10 mM and autoclaved gelatin to 100 μg/ml, an excess of HpaII restriction endonuclease was added and the mixture was incubated 1 h at 37 C. DNA was mounted for electron microscopy, and contour lengths were measured as previously described (9). The HpaII endonuclease was purified by a modification of the second method described by P. A. Sharp, B. Sugden, and J. Sambrook (Biochemistry, in press).

Figure 2 shows the histogram of DNA lengths from the midpoint of the denaturation loop to the nearest end. Of 33 linear molecules containing T4 gene 32 protein that were scored by electron microscopy, 88% contained loops between 0.25 and 0.29 SV40 fractional length from

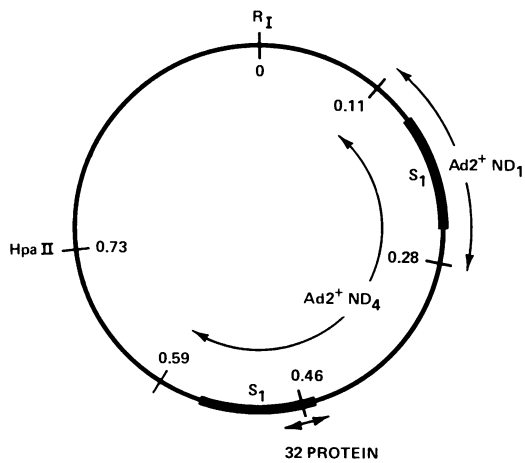

FIG. 1. *Map of SV40 DNA. The R_I restriction endonuclease cleavage site is the origin. Positions are expressed in SV40 DNA fractional length from the origin. The SV40 segment of Ad2+ND$_4$ induces all known early SV40 antigens and early SV40 RNA sequences (7, 8, 10). The regions of cleavage by single-strand-specific endonuclease S_1 are 0.45 to 0.55 (predominant at high ionic strength) and 0.15 to 0.25 (4).*

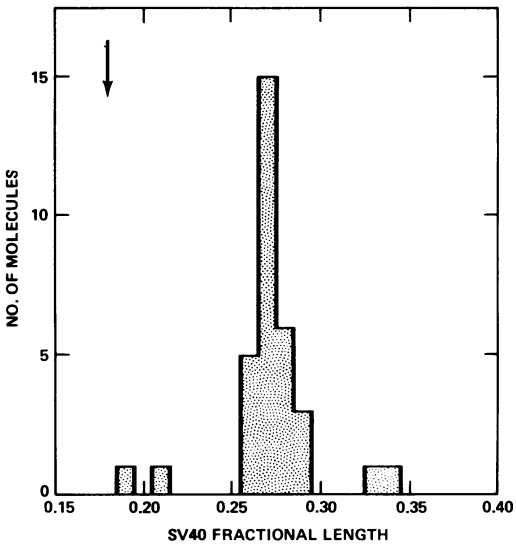

FIG. 2. *HpaII endonuclease cleavage of SV40(I) DNA-T4 gene 32 protein complexes. Distance from the midpoint of the denaturation loop to the nearer of the two ends is presented as a fraction of the length of circular SV40 DNA. The arrow indicates the expected position of the peak if the denaturation loop midpoint were 0.55 on the SV40 DNA map. The mean total length of 33 molecules was 0.976 fractional length, standard deviation 0.040. Lengths were corrected to unit length by addition of 0.012 to each end because we have found that SV40(L_{HpaII}) DNA is 0.03 ± 0.005 shorter than SV40(II) DNA, and this difference is removed by phenol extraction.*

an end (mean 0.27); the arrow shows the position on the histogram at which denaturation loops located at 0.55 SV40 map position would have occurred. The mean length of the denaturation loops was 0.042 fractional length (standard deviation 0.008). We conclude from this experiment that the T4 gene 32 protein binds predominantly to the region of SV40 DNA corresponding to 0.44 to 0.48 SV40 map unit.

If SV40 DNA with bound gene 32 protein can be denatured and renatured with other SV40 DNA strands, the 32 protein loop will be a useful marker for heteroduplex experiments. However, the usual method involving glutaraldehyde fixation for electron microscopy of DNA with 32 protein denaturation loops apparently cross-links the DNA strands. The HpaII-cleaved linear molecules with bound gene 32 protein were exposed to 0.1 N NaOH for 10 min at 25 C, and then chilled to 0 C, neutralized, and immediately mounted for electron microscopy as above. Most linear molecules that were incubated with glutaraldehyde but did not have 32 protein loops were denatured. However, the two strands of the 32 protein denaturation loops remained attached to each other, and the remainder of the strands attached to the loops were in duplex form.

SV40 covalently closed superhelical DNA can be cleaved by S_1 nuclease, a single-strand specific endonuclease (2, 12), to yield unit length linear molecules with no internal nicks (4). At high ionic strength (250 mM NaCl), the cleavage occurs preferentially in one region: 0.45 to 0.55 on the SV40 map. We surmise, therefore, that in SV40(I) DNA, this region is partially denatured or can be easily denatured. Denaturation mapping by electron microscopy has revealed that the same region is the most readily denaturable at elevated pH (11). Although a high AT content in this region would be a plausible explanation for the denaturability at high pH and the localized unwinding due to destabilization induced by superhelicity (3), other structural features should not be discounted.

This work was supported in part by Public Health Service research grant GM-13235 from the National Institute of General Medical Sciences and research grant VC 23A from the American Cancer Society. J. F. M. is a Public Health Service Trainee, training grant 5 TI GM-0196.

LITERATURE CITED

1. Alberts, B. M., and L. Frey. 1970. T4 bacteriophage gene 32: a structural protein in the replication and recombination of DNA. Nature (London) **227**:1313-1318.
2. Ando, T. 1966. A nuclease specific for heat-denatured DNA isolated from a product of Aspergillus oryzae. Biochim. Biophys. Acta **114**:158-168.
3. Bauer, W., and J. Vinograd. 1970. Interaction of closed circular DNA with intercalative dyes. II. The free energy of superhelix formation in SV40 DNA. J. Mol. Biol. **47**:419-435.
4. Beard, P., J. F. Morrow, and P. Berg. 1973. Cleavage of circular, superhelical Simian Virus 40 deoxyribonucleic acid to a linear duplex by S_1 nuclease. J. Virol. **12**:1303-1314.
5. Delius, H., N. J. Mantell, and B. Alberts. 1972. Characterization by electron microscopy of the complex formed between T4 bacteriophage gene 32-protein and DNA. J. Mol. Biol. **67**:341-350.
6. Hedgpeth, J., H. M. Goodman, and H. W. Boyer. 1972. DNA nucleotide sequence restricted by the RI endonuclease. Proc. Nat. Acad. Sci. U.S.A. **69**:3448-3452.
7. Kelly, T. J., Jr., and A. M. Lewis, Jr. 1973. Use of nondefective adenovirus-simian virus 40 hybrids for mapping the simian virus 40 genome. J. Virol. **12**:643-652.
8. Levine, A. S., M. J. Levin, M. N. Oxman, and A. M. Lewis, Jr. 1973. Studies of nondefective adenovirus2-simian virus 40 hybrid viruses. VII. Characterization of the simian virus 40 ribonucleic acid species induced by five nondefective hybrid viruses. J. Virol. **11**:672-681.
9. Morrow, J. F., and P. Berg. 1972. Cleavage of Simian Virus 40 DNA at a unique site by a bacterial restriction enzyme. Proc. Nat. Acad. Sci. U.S.A. **69**:3365-3369.
10. Morrow, J. F., P. Berg, T. J. Kelly, Jr., and A. M. Lewis, Jr. 1973. Mapping of Simian Virus 40 early functions on the viral chromosome. J. Virol. **12**:653-658.
11. Mulder, C., and H. Delius. 1972. Specificity of the break produced by restricting endonuclease R_I in Simian Virus 40 DNA, as revealed by partial denaturation mapping. Proc. Nat. Acad. Sci. U.S.A. **69**:3215-3219.
12. Vogt, V. 1973. Purification and further properties of single-strand-specific nuclease from *Aspergillus oryzae*. Eur. J. Biochem. **33**:192-200.