Proc. Nat. Acad. Sci. USA
Vol. 72, No. 3, pp. 989–993, March 1975

Biochemical Method for Mapping Mutational Alterations in DNA with S1 Nuclease: The Location of Deletions and Temperature-Sensitive Mutations in Simian Virus 40*

(restriction endonucleases/single-strand cleavage)

THOMAS E. SHENK, CARL RHODES, PETER W. J. RIGBY, AND PAUL BERG

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Contributed by Paul Berg, January 6, 1975

ABSTRACT **S1 nuclease (EC 3.1.4.X), a single-strand-specific nuclease, can be used to accurately map the location of mutational alterations in simian virus 40 (SV40) DNA. Deletions of between 32 and 190 base pairs, which are at or below the limit of detectability by conventional electron microscopic analysis of heteroduplex DNAs, have been located in this way. To map a deletion, a mixture of unit length, linear DNA, prepared from the SV40 deletion mutant and its wild-type parent, are denatured and reannealed to form heteroduplexes. S1 nuclease can cut such heteroduplexes at the nonbase-paired region to produce fragments whose lengths correspond to the position of the deletion. Similarly, specific fragments are produced when S1 nuclease cleaves a heteroduplex formed from the DNAs of SV40 temperature-sensitive mutants and either their revertants or wild-type parents. Thus, the positions of the nonhomology between these DNAs can be determined.**

S1 nuclease (EC 3.1.4.X) from *Aspergillus oryzae* degrades single- but not double-stranded DNA (1); nevertheless, superhelical simian virus 40 (SV 40) (2) and polyoma (3) DNAs are converted to unit length linear molecules by this enzyme. Presumably, this occurs because unpaired, or weakly hydrogen-bonded regions, susceptible to S1 nuclease, occur or can be induced in the strained superhelical molecule. Since nicked circular molecules appeared to be intermediates in the double-strand cleavage, we surmised that the enzyme was capable of cleaving the intact strand opposite or near the nick (2).

This property suggested that S1 nuclease could be used to map the location of small deletions, insertions, or, in fact, any difference in base sequence between otherwise homologous DNAs. For example, heteroduplex DNA molecules formed from the complementary strands of a deletion mutant and wild-type SV40 DNA contain a single-strand loop at a position corresponding to the deletion (Fig. 1). If S1 nuclease can digest this single-strand loop and subsequently cleave the intact strand at the nick (or gap), fragments whose length corresponds to the position of the deletion loop should be generated. In principle, insertion mutations could also be located this way.

Here we report that S1 nuclease can indeed be used to locate a deletion of as few as 32 base pairs. Moreover, S1 nuclease also cleaves heteroduplex molecules formed from the DNAs of SV40 temperature-sensitive mutants and either their revertants or wild-type parents. If, as is likely, the base sequence of the temperature-sensitive mutant DNA differs from the wild-type or revertant DNA by a single base change, this method could be useful for accurately mapping point mutations, thereby making reliance on genetic recombination unnecessary.

Abbreviation: SV40, simian virus 40.

* Many of these experiments were reported at the Cold Spring Harbor Symposium on Quantitative Biology, June, 1974.

MATERIALS AND METHODS

Cells and Viruses. The origin and the procedures for the growth of primary African green monkey kidney cells (AGMK) and the established monkey kidney cell lines (CV-1P and MA-134) have been described (4). All virus stocks and virus DNA were prepared in MA-134 cells. Plaque assays were performed on CV-1P cells. Mutants *dl*-808 and *dl*-861 are viable deletion mutants of SV40 (5, 6). SV40 mutants *tsA*30 and *tsD202*, as well as their wild-type parents, were obtained from Peter Tegtmeyer (7) and Robert Martin (8), respectively. Temperature-sensitive mutants were grown at 32°. Spontaneous revertants of these mutants were from plaques that arose on cell monolayers incubated at 41°.

Enzymes. S1 nuclease was prepared from "Enzopharm" powder (Enzyme Development Corp., New York) by the procedure of Vogt (9) with minor modifications. One unit of S1 nuclease releases 1.0 nmol of nucleotides per min at 37° when acting on sonicated, denatured salmon sperm DNA at pH 4.4 in the presence of 0.5 mM Zn^{++} and 280 mM Na^{+}. S1 nuclease obtained from a commercial source (Miles Laboratories, Inc.) was also found to give good results in the types of experiments reported here, although the "nibbling" effect (see *Results*) was greater. *Eco*R1 restriction endonuclease was prepared by the procedure of Greene *et al.* (10). *Hpa*I and *Hpa*II restriction endonucleases were prepared essentially by the procedure of Sharp *et al.* (11).

DNA Substrates. SV40 DNA was extracted according to Hirt (12) from MA-134 cells infected at a multiplicity of <0.05 PFU per cell when >90% of the cells showed cytopathic effect. Covalently closed viral DNA [SV40 (I)] was purified directly from the supernatant by adding CsCl to 1.56 g/cm^3 and ethidium bromide to 200 μg/ml and centrifuging to equilibrium. The band of SV40 (I) DNA was collected, and the ethidium bromide was removed by passing the DNA through Dowex-50 (13). Full length linear DNA or fragments derived from it were prepared with restriction endonucleases using published protocols (11, 14), and then the DNA was repurified by velocity sedimentation in a neutral CsCl gradient.

Cleavage of Heteroduplex DNA with S1 Nuclease. Heteroduplexes of *Eco*RI or *Hpa*II endonuclease-generated linear

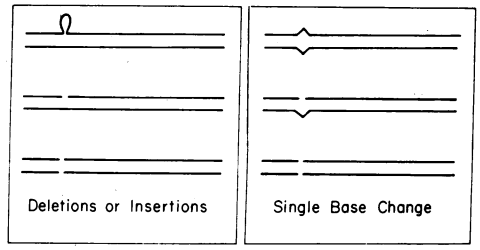

FIG. 1. Putative cleavages of heteroduplexes which contain mismatched regions susceptible to S1 nuclease. A duplex DNA with a single nick (middle) is proposed as an intermediate in the S1 nuclease cutting reaction. There is as yet no direct evidence for such an intermediate.

SV40 DNAs were prepared by denaturing a mixture containing equal parts of two DNA species (1.5–6 μg/ml of each) in NaOH (0.1 N). After 10 min at room temperature, the solution was titrated to pH 7–8 with HCl, the Na^+ concentration was raised to 300 mM, and the DNA was reannealed at 68° for 3 min. The reannealed DNA was treated with S1 nuclease (135 units/μg of DNA) in the presence of Zn^{++} (4.5 mM), Na^+ (280 mM), and CH_3COO^- (30 mM) at pH 4.4. After 30 min at 25°, the reaction was terminated by raising the pH with 0.05 volume of Tris base (2 M), and by increasing the Na^+ concentration to 500 mM. To decrease the volume and Na^+ concentration of the sample prior to gel electrophoresis, the DNA was precipitated at −20° after the addition of yeast RNA (20 μg/ml) and 2 volumes of ethanol.

In our earlier experiments there was some cleavage by S1 nuclease of homoduplex DNA within A+T-rich regions. To minimize the technical difficulties caused by this background, we sought digestion conditions that would reduce or randomize the location of such cleavages. Under the conditions we adopted (4.5 mM Zn^{++}, 280 mM Na^+), the difference in T_m between two DNAs of substantially different base composition is eliminated (Table 1). In 4.5 mM Zn^{++}, *Micrococcus luteus* (72% G+C) and salmon sperm (43% G+C) DNAs melt at the same temperature (T_m) and over a narrower temperature range (ΔT) than was the case with our earlier conditions (0.5 mM Zn^{++}). With these reaction conditions inadvertent cleavages are nearly random and, therefore, any fragments produced are more evenly distributed throughout the analytical electrophoresis gels.

Gel Electrophoresis. Agarose gels (1.2%, 6 mm in diameter, 200 mm long) were prepared in Tris–borate buffer (89 mM Tris-OH, 89 mM boric acid, 2.5 mM EDTA, pH 8.2) (10). Samples were applied in 60 μl of Tris–borate buffer containing sucrose (20% w/v). Electrophoresis was at 40 V for 17 hr. The DNA bands were stained with ethidium bromide and visualized using a short wavelength ultraviolet light. The fluorescent bands were photographed using a Vivitar orange (02) filter and Kodak TX-135 film. The negatives were scanned with a Joyce, Loebl and Co. microdensitometer.

RESULTS

S1 Nuclease Cleaves Duplex DNA at the Site of a Single-Stranded Nick. Essential to our approach of mapping deletions is the predicted ability of S1 nuclease to digest the single-stranded loop of a deletion heteroduplex and to cleave the resulting nicked or gapped duplex structure at the interruption (see Fig. 1). The first expectation seemed plausible from the known activity of the enzyme (1), but, although earlier experiments (2) had indicated that S1 nuclease could carry out the second step, we sought more convincing evidence of this ability. Accordingly, SV40 DNA nicked once in either of its two strands at the *Hpa*II endonuclease restriction site (located 0.735 SV40 fractional length clockwise from the *Eco*RI endonuclease-cleavage site), was converted to linear DNA by cleavage with *Eco*RI endonuclease. These molecules migrate in agarose gels as unit length linear SV40 DNA (Fig. 2A). This uniquely nicked linear DNA is cleaved by S1 nuclease to form two fragments, 0.26 and 0.73 SV40 fractional length (Fig. 2B), measured from the mobility of fragments of known size (Fig. 2C). Because S1 nuclease appears to "nibble" the ends of duplex DNA molecules during the course of the reaction, the length of the fragments is slightly underestimated. The extent of "nibbling" is difficult to determine accurately but is about 30 base pairs or 0.006 SV40 fractional length. Applying this correction, it is clear that the nicked DNA molecules have been cleaved by S1 nuclease at or very near 0.735, the map coordinate of the *Hpa*II endonuclease restriction site.

TABLE 1. *Effect of Zn^{++} on melting transitions of DNA*

Buffer	*M. luteus* DNA		Salmon sperm DNA	
	T_m, °	ΔT, °	T_m, °	ΔT, °
S1 nuclease reaction buffer containing 0.5 mM Zn^{++}	85.8	1.7	77.5	2.8
S1 nuclease reaction buffer containing 4.5 mM Zn^{++}	74.5	0.8	74.2	1.6

Micrococcus luteus and salmon sperm DNAs were heated at approximately 1°/min in the indicated buffers. Temperature was measured with a thermocouple in a cuvette in the sample changer, and melting transitions were monitored at 260 nm with a Gilford model 2000 spectrophotometer. T_m is the melting temperature and ΔT is the breadth of the transition from 1/4 to 3/4 maximum hyperchromicity.

S1 Nuclease Cleaves Heteroduplex DNAs at the Site of Deletion Loops. Either naturally arising (5) or biochemically generated (6) mutants of SV40 with deletions of the *Hpa*II endonuclease cleavage site are suitable model substrates for testing the validity of the mapping procedure. DNAs from mutants *dl*-808 and *dl*-861, which contain deletions of about 190 and 32 base pairs, respectively (0.035 and 0.007 SV40 fractional length), as well as wild-type DNA were converted to unit length linear DNAs by digestion with *Eco*RI endonuclease. Electron microscopic examination of heteroduplexes formed from *dl*-808 and wild-type DNA showed a barely detectable denaturation loop 0.245 SV40 fractional length from the nearest *Eco*RI endonuclease generated end (5). Cleavage of these same heteroduplex structures with S1 nuclease produced two fragments of about 0.73 and 0.24 SV40 fractional length (15). Thus, S1 nuclease can digest the single-stranded denaturation loop (0.035 SV40 fractional length) and cleave the heteroduplex at that point. The deletion loop in heteroduplexes formed from *dl*-861 and wild-type DNA is too small to be seen in the electron microscope; nevertheless, these molecules are readily cleaved into two fragments whose lengths are 0.74 and 0.26 SV40 fractional length (corrected for the "nibbling" effect mentioned above)

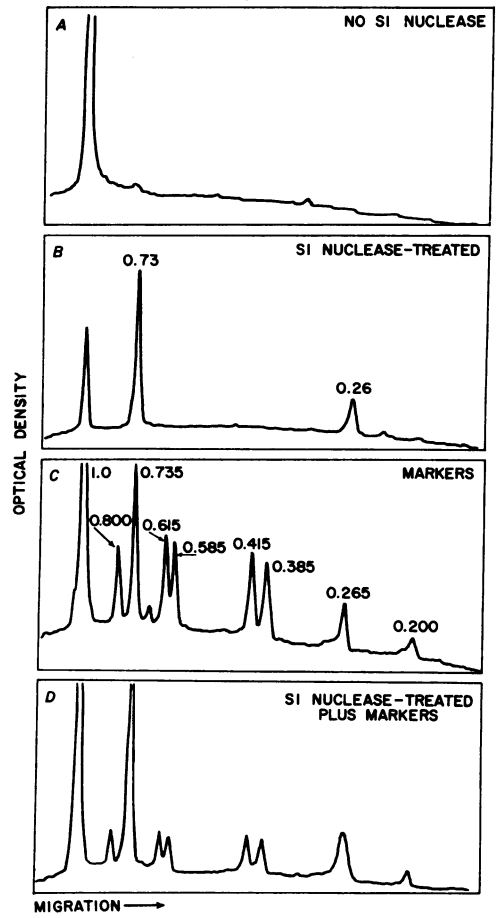

FIG. 2. Cleavage by S1 nuclease of *Eco*R1 endonuclease-generated linear SV40 DNA containing a single-strand nick at the *Hpa*II restriction site. Relaxed circular SV40 (II) DNA, containing a specific nick at the *Hpa*II restriction site, was isolated from a reaction in which *Hpa*II endonuclease had converted approximately 50% of the input SV40 (I) DNA to SV40 (II) and SV40 linear (L) DNAs. The final yield of SV40 (II) DNA was 12% of the input SV40 (I) DNA. This SV40 (II) DNA (5 μg/ml), containing a single-strand nick at the *Hpa*II endonuclease site, was cleaved to linear molecules with *Eco*RI endonuclease, and then treated with S1 nuclease. Aliquots of the digest (0.2 μg of DNA) were applied to gels. (*A*) Untreated, nicked, linear DNA. (*B*) S1 nuclease-treated nicked, linear DNA. (*C*) Marker fragments alone. These include *Eco*RI endonuclease-generated SV40 linear DNA, fragments obtained by sequential cleavage of SV40 DNA with *Hpa*II and *Eco*RI endonucleases, and fragments obtained by partial cleavage of SV40 DNA with *Hpa*I endonuclease. (*D*) Same as B with marker fragments (0.15 μg) added. Numbers are the length of the fragments relative to intact linear SV40 DNA.

(Fig. 3B). Since a mixture of the corresponding homoduplexes, formed by denaturation and renaturation of each DNA by itself, does not yield such fragments after S1 nuclease digestion (Fig. 3A), we may conclude that S1 nuclease can detect and cleave a duplex DNA, preferentially, at small single-stranded loops, and thereby permit the accurate mapping of small deletions, insertions, and very likely, substitutions.

S1 Nuclease Cleaves Heteroduplexes Formed Between Wild-Type, Temperature-Sensitive, and Revertant DNAs. Since S1 nuclease could cleave heteroduplexes at the position of

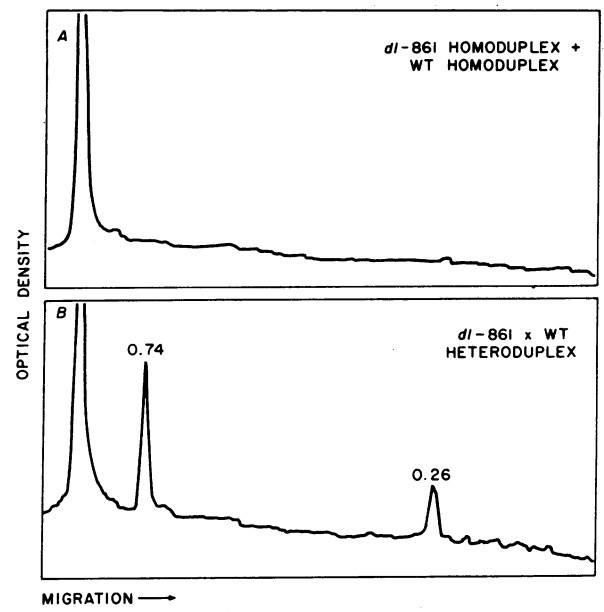

FIG. 3. Cleavage by S1 nuclease of the heteroduplex prepared from *Eco*R1 endonuclease-generated linear DNAs of *dl*-861 and its wild-type (WT) parent. The S1 nuclease reactions contained 5 μg/ml of DNA. Samples of 0.2 μg of DNA were applied to each gel. (*A*) S1 nuclease-treated homoduplexes. (*B*) S1 nuclease-treated heteroduplexes formed from *dl-861* and wild-type DNAs.

small single-stranded loops, we wished to test whether the enzyme could cleave heteroduplex DNA at mismatches due to single base differences. Lacking DNAs with defined single base changes, we examined the susceptibility of heteroduplexes formed from *Eco*RI endonuclease-cleaved temperature-sensitive (ts), revertant (−R) and wild-type SV40 DNAs. Both *tsA30* and *tsD202* were induced by hydroxylamine and though the nature and number of changes in the base sequence are not known, they are probably single base changes.

Because the DNA of *tsA30* and its revertant, *tsA30*-R, were expected to have the lowest number of base pair differences, we examined the S1 nuclease susceptibility of their heteroduplex first. As expected, the mixed homoduplexes of *Eco*RI endonuclease-cut mutant and revertant DNAs did not yield specific fragments during S1 nuclease digestion (Fig. 4A). But the heteroduplex of the temperature-sensitive and revertant DNA was cut to produce two easily discernible fragments of 0.68 and 0.32 SV40 fractional length (Fig. 4B). This places the mismatch between *tsA30* and its revertant at either 0.32 or 0.68 on the SV40 map. These two alternatives could be distinguished by examining the S1 nuclease cleavage products of the corresponding heteroduplex formed from *Hpa*II endonuclease-cleaved mutant and revertant DNA. In this instance the mismatch would be expected to be in a different location relative to the ends of the heteroduplex. Since S1 nuclease cleaved these heteroduplex DNAs, generating fragments of 0.59 and 0.42 SV40 fractional length (data not shown), the mismatch between *tsA30* and its revertant must be located at 0.32 on the SV40 map.

The analysis of the S1 nuclease cleavage products of the heteroduplex between *tsA30* and wild-type DNA revealed at least one mismatched sequence (Fig. 4C). Cleavage of the heteroduplex formed from *Eco*RI endonuclease-generated linear *tsA30* and wild-type DNA with S1 nuclease yielded

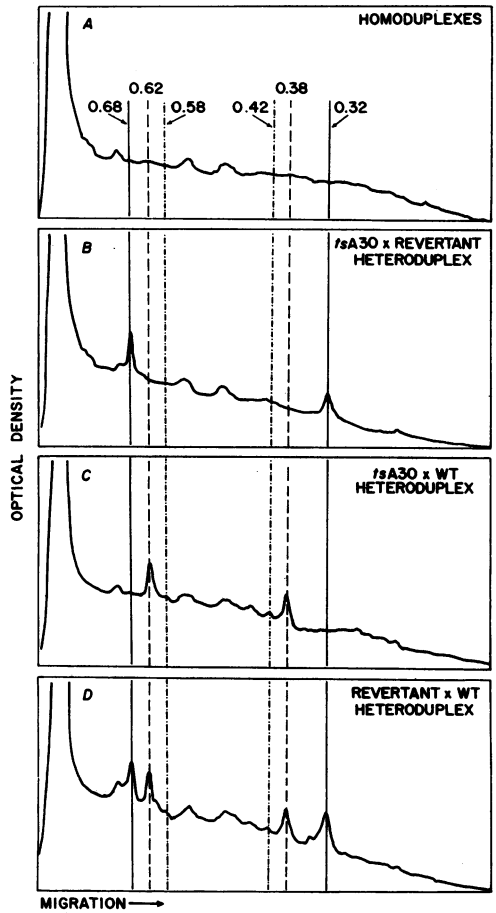

FIG. 4. Cleavage by S1 nuclease of heteroduplexes prepared from *Eco*RI endonuclease-generated linear DNAs containing putative single base mismatches. The S1 nuclease reactions contained 10 μg/ml of DNA. Samples of 0.7 μg of DNA were applied to each gel. The vertical lines drawn through the DNA bands serve to match pairs of fragments whose sizes add up to unit length SV40 DNA. (*A*) S1 nuclease-treated homoduplexes. (*B*) S1 nuclease-treated heteroduplexes formed from *tsA30* and *tsA30*-R DNAs. (*C*) S1 nuclease-treated heteroduplexes formed from *tsA30* and wild-type DNAs. (*D*) S1 nuclease-treated heteroduplexes formed from *tsA30-R* and wild-type DNAs.

predominantly fragments of 0.62 and 0.38 SV40 fractional length. In several earlier experiments, carried out under different digestion conditions, S1 nuclease produced another, apparently specific, cleavage which yielded small quantities of fragments of 0.58 and 0.42 SV40 fractional length (15). Only a hint of such a cleavage is seen with the present reaction conditions (Fig. 4C). Possibly the earlier result was spurious and there is indeed only one S1 nuclease-sensitive mismatch in the heteroduplex of *tsA30* and wild-type DNA. Alternatively, there is more than one mismatch in the heteroduplex of *tsA30* and wild-type DNA and only the site that yields the fragments of 0.62 and 0.38 SV40 fractional length is appreciably cleaved by S1 nuclease.

A decision as to whether the mismatch occurs at 0.38 or 0.62 on the SV40 map was made, as mentioned earlier, using analogous heteroduplex molecules generated from *Hpa*II endonuclease-cut *tsA30* and wild-type DNA; the mismatch can be assigned to 0.38 map position (data not shown).

Heteroduplexes produced from *tsA30*-R and wild-type DNAs yield two specific classes of fragments after cleavage

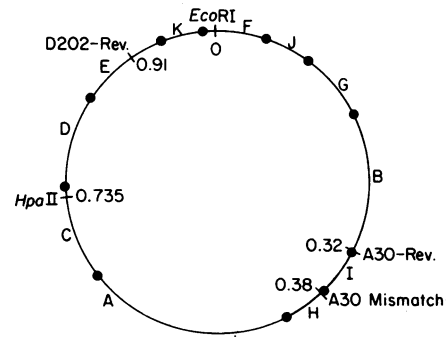

FIG. 5. The locations of point mutations on the SV40 chromosome as determined by the S1 nuclease procedure. The solid circles on the map represent points at which *Hind* II + III endonucleases cut, and the letters designate the fragments produced by this cleavage (17).

with S1 nuclease (Fig. 4D). One specific cleavage generated the same two fragments as those obtained from the heteroduplex prepared from *tsA30* and wild-type DNA; the other cleavage yielded the two fragments previously obtained with the heteroduplex prepared from *tsA30* and *tsA30*-R DNA.

Thus, *tsA30* contains at least one alteration relative to its wild-type parent; this occurs at 0.38 on the SV40 map (Fig. 5). The alteration causing reversion of the *tsA30* phenotype is, therefore, a second-site mutation which occurred at 0.32 on the SV40 map. This indicates that the map coordinates 0.32 to 0.38, which is the region within which the mutant and second site revertant differ from wild-type, lie within the *A* complementation group of SV40. This conclusion is reinforced by the findings of Lai and Nathans (16), who have determined by marker rescue experiments that the *tsA30* mutation is located within *Hind* II + III fragment H (0.375–0.43 map position) and the *tsA28* mutation (another *tsA* allele) occurs within *Hind* II + III fragment I (0.325–0.375 map position) (see Fig. 5). Additional experiments with other *tsA* mutants or *A* mutants resulting from deletions and insertions should better define the physical limits of the *A* cistron.

The S1 nuclease mapping procedure has also been used to locate a second SV40 cistron. Heteroduplex molecules formed from *tsD202* and a spontaneous revertant, *tsD202*-R, were digested with S1 nuclease as indicated above (data not shown). A fragment 0.91 SV40 fractional length was produced by S1 nuclease cleavage of the heteroduplex formed from *Eco*RI endonuclease-cut DNAs and a fragment 0.82 SV40 fractional length from the heteroduplex of *Hpa*II endonuclease-cut DNAs. These results are consistent with the existence of a nonhomology between *tsD202* and its revertant at 0.91 on the SV40 map.

In this instance also, the reversion does not restore the wild-type sequence. This conclusion follows from the finding that though S1 nuclease can cleave the heteroduplex formed from *tsD202*-R and wild-type DNA, it fails to cleave the heteroduplex generated from *tsD202* and wild-type DNA. Perhaps the particular mismatch generated in the heteroduplex mode from *tsD202* and wild-type DNA cannot be readily "recognized" or cleaved by S1 nuclease.

Here, too, the data of Lai and Nathans (16) substantiate our assignment of the *D* cistron to a region of the physical map containing the coordinate 0.91. They have found that *Hind* II + III fragment E (map position 0.86–0.945) contains the sequence modified by the *tsD202* mutation.

DISCUSSIONS

There seems little doubt that S1 nuclease can be used to map deletions and insertions (or, in fact, any change that produces single-strand segments in heteroduplex structures) in DNA. As with electron microscopic examination of heteroduplexes, the method permits accurate analysis of the location and size of these and other gross perturbations of DNA structure. Of particular importance, however, is the ability of the S1 nuclease-mapping procedure to detect and locate deletions and insertions that are too small to be seen by electron microscopy.

A most promising but still provisional conclusion is that S1 nuclease can detect and map single base changes as well. We are presently cautious because, though reasonable, it is still unproven that the temperature-sensitive DNAs differ from their revertant or wild-type DNAs by only single bases at the sites where S1 nuclease acts. Hopefully, experiments with DNAs having known base changes at specific locations can resolve this uncertainty.

Our limited experience with the method has already indicated several shortcomings. Under the conditions used, cleavage of heteroduplexes with putative single base mismatches is slower and more limited in extent than that observed with deletion and insertion heteroduplexes. The rate-limiting step in this cleavage is the introduction of the first nick at the site of the mismatch, since molecules containing a single-stranded scission are quite efficiently cleaved by the nuclease (Fig. 2). As a consequence of the limited cleavage at the site of possible single base mismatches, the signal (specific cutting at the mismatch) to noise (random cutting of the heteroduplex DNA) ratio is low. Several parameters may be tested to deal with this problem: the conditions required to ensure perfect reannealing in the formation of the heteroduplex substrates for S1 nuclease; the conditions that minimize the existence of regions of even transient single-strandedness (e.g., reaction conditions that eliminate or randomize transient single-strandedness due to differences in base composition and sequence, Table 1); the efficiency of S1 nuclease cleavage of mismatches involving different base pairs occurring within different sequences (e.g., A/G versus T/C and the influence of neighboring stretches of high or low G+C content). A collateral problem related to the noise or nonspecific cleavages is the possibility that sequence heterogeneity can result during propagation of a supposedly homogeneous DNA. With increasing experience we should also be better able to assign error limits to the lengths of the fragments and, therefore, to obtain more precise assignments of the map position of mutations.

Even at its present stage of development, the S1 nuclease procedure has permitted us to map several changes in the *A* and *D* cistrons of SV40 (Fig. 5). For example, at least one site (0.38 on the SV40 map) distinguishes the base sequence of *tsA30* and its wild-type parent. Only one difference was detected between the sequence in *tsA30* and its revertant *tsA30*-R, and that occurs at 0.32 on the map. Consistent with this is the fact that *tsA30*-R differs from the wild-type at a minimum of two map locations: 0.38 and 0.32. Thus, the revertant mutation has occurred at a second site. Although we could infer the existence of a region of nonhomology at map position 0.91 between *tsD202* and *tsD202*-R and between *tsD202*-R and wild-type DNAs, we have been unable to detect a mismatch in the heteroduplex formed between *tsD202* and wild-type DNA. The reason for this is unknown and further experiments are needed.

Some mention should be made of several ramifications of the method. First, it provides an approach to genetic mapping in systems where genetic recombination is lacking or difficult to measure. Second, the procedure enables one to locate genotypic alterations that cause no detectable change in phenotype. Third, the ability of S1 nuclease to cleave a duplex DNA at a mismatch may make it possible to isolate discrete segments of a genome if that segment can be bounded by small deletions. For example, a heteroduplex between two different deletion mutants should yield three fragments after S1 nuclease cleavage, one of which contains the segment between the two mutations.

This work was supported in part by research grants from the U.S. Public Health Service (GM-13235-09) and the American Cancer Society (VC-23C). T.E.S. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. C.R. was supported by funds from the California Division of the American Cancer Society. P.W.J.R. is a Fellow of the Helen Hay Whitney Foundation.

1. Ando, T. (1966) "A nuclease specific for heat-denatured DNA isolated from a product of *Aspergillus oryzae*," *Biochim. Biophys. Acta* **114**, 158–168.
2. Beard, P., Morrow, J. F. & Berg, P. (1973) "Cleavage of circular, superhelical SV40 DNA to a linear duplex by S1 nuclease," *J. Virol.* **12**, 1303–1313.
3. Germond, J. E., Vogt, V. M. & Hirt, B. (1974) "Characterization of the single-strand-specific nuclease S1 activity on double-stranded supercoiled polyoma DNA," *Eur. J. Biochem.* **43**, 591–600.
4. Mertz, J. E. & Berg, P. (1974) "Defective SV40 genomes: Isolation and growth of individual clones," *Virology* **62**, 112–124.
5. Mertz, J. E. & Berg, P. (1974) "Viable deletion mutants of simian virus 40: Selective isolation by means of a restriction endonuclease from *Hemophilus parainfluenzae*," *Proc. Nat. Acad. Sci. USA* **71**, 4879–4883.
6. Carbon, J., Shenk, T. E. & Berg, P. (1975) "A simple biochemical procedure for the production of small deletions in SV40 DNA," *Proc. Nat. Acad. Sci. USA*, in press.
7. Tegtmeyer, P. (1972) "SV40 DNA synthesis: the viral replicon," *J. Virol.* **10**, 591–598.
8. Chou, J. Y. & Martin, R. G. (1974) "Complementation analysis of SV40 mutants," *J. Virol.* **13**, 1101–1109.
9. Vogt, V. M. (1973) "Purification and further properties of single-strand-specific nuclease from *Aspergillus oryzae*," *Eur. J. Biochem.* **33**, 192–200.
10. Greene, P. J., Betlach, M. C., Goodman, H. M. & Boyer, H. W. (1974) "The *Eco*RI restriction endonuclease," in *Methods in Molecular Biology*, ed. Wickner, R. B. (Marcel Dekker, Inc., New York), pp. 87–111.
11. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) "Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose–ethidium bromide electrophoresis," *Biochemistry* **12**, 3055–3063.
12. Hirt, B. (1967) "Selective extraction of polyoma DNA from infected mouse cell cultures," *J. Mol. Biol.* **26**, 365–369.
13. Radloff, R., Bauer, W. & Vinograd, J. (1967) "A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: The closed circular DNA in HeLa cells," *Proc. Nat. Acad. Sci. USA* **57**, 1514–1521.
14. Mertz, J. E. & Davis, R. W. (1972) "Cleavage of DNA by R1 restriction endonuclease generates cohesive ends," *Proc. Nat. Acad. Sci. USA* **69**, 3370–3374.
15. Shenk, T. E., Rhodes, C., Rigby, P. W. J. & Berg, P. (1975) "Mapping of mutational alterations in DNA with S1 nuclease: The location of deletions, insertions and temperature-sensitive mutations in SV40," *Cold Spring Harbor Symp. Quant. Biol.* **39**, 61–67.
16. Lai, C. J. & Nathans, D. (1974) "Mapping temperature-sensitive mutants of SV40: Rescue of mutants by fragments of viral DNA," *Virology* **60**, 466–475.
17. Danna, K. J., Sack, G. H. & Nathans, D. (1973) "A cleavage map of the SV40 genome," *J. Mol. Biol.* **78**, 363–376.