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Biochemistry

A site-specific single-strand endonuclease from the eukaryote *Chlamydomonas**

(DNA/endodeoxyribonuclease/enzymatic cleavage)

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ABSTRACT **We have found a unique deoxyribonuclease in extracts of the eukaryotic green alga *Chlamydomonas*. When incubated with viral DNA from adenovirus-2, this enzyme produces discrete fragments that form bands upon electrophoresis in an agarose gel. Site specificity of the enzymatic cleavage, examined by identifying the 5′-terminal nucleotides in cleaved adenovirus-2 DNA and by studies with synthetic polynucleotides of defined sequence, indicates that the initial endonucleolytic cleavage occurs at a site containing a deoxythymidine residue. Electron microscopy of cleaved adenovirus-2 DNA revealed single-strand segments within duplex DNA. We propose that the enzyme acts by making initial site-specific single-strand incisions, followed by subsequent excision on the same strand, producing a gapped duplex molecule; and that double-strand scissions result from limited occurrence of overlapping single-strand gaps on complementary strands.**

Specific endonucleases are important components of DNA metabolism in both prokaryotes and eukaryotes. For example, endonucleases that detect structural distortions in a DNA helix and remove mismatched bases have been implicated in DNA repair (1) and gene conversion (2). In bacteria, modification-restriction systems consist of site-specific DNA methylases that protect sites by methylation and the corresponding endonucleases that recognize the same sites and cleave unmethylated DNA (3). In eukaryotes, a wide range of biological processes, such as the selective silencing of particular DNAs (4) and certain aspects of differentiation (5), have been postulated to utilize specific methylases and endonucleases in restriction-modification processes analogous to those of bacteria, but no such enzymes have been described.

In the sexual alga *Chlamydomonas*, inheritance of the chloroplast genome is non-Mendelian (maternal) as a result of the selective degradation of the chloroplast DNA of paternal origin that occurs in zygotes (6, 7); we have postulated that the molecular mechanism of this degradation is a modification-restriction process (6, 7). In vegetative cells, the chloroplast genes undergo nonreciprocal recombination events at a high frequency (8, 9), suggesting that the molecular events may involve specific nucleases of the kind described in fungi (2, 10). Thus, the genetic evidence, suggesting that the chloroplast DNA of *Chlamydomonas* is acted upon by an array of site-specific endonucleases, led us to examine the endonucleolytic activities of this organism.

We report here the discovery and preliminary characterization of site-specific endonucleolytic activity in a eukaryote. This enzymatic activity, in extracts of *Chlamydomonas*, produces single-strand gaps starting at specific sites in homoduplex DNA. The gap-forming ability of this nuclease is unique in that the initial cleavage as well as subsequent development of the gap may be due to the action of a single enzymatic moiety.

Abbreviation: Ad-2, adenovirus-2.

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MATERIALS AND METHODS

The enzyme was obtained from vegetative cells of *Chlamydomonas reinhardi*, strain 21 gr, mating type plus. Cells were grown in liquid culture under continuous light on acetate-supplemented minimal medium (11) at 25°, harvested in late logarithmic phase of growth (6 to 7 × 10^6 cells per ml), washed twice with ice-cold 10 mM 2-mercaptoethanol/10 mM Tris·HCl (pH 7.9), and stored as a pellet at −70°.

Isolation of Endonuclease. Five to ten grams of frozen cells were thawed and resuspended in two volumes of cold extraction buffer (10 mM 2-mercaptothanol/10 mM Tris·HCl, pH 7.9). All subsequent steps were at 2°. The cells were disrupted by sonication (12 times for 30 sec each) and the sonicated cells were centrifuged at 100,000 × *g* for 90 min. The supernatant was adjusted to 1 M NaCl, and was applied to a column of Sephadex G-100 (100 × 2.5 cm) equilibrated with 1 M NaCl/10 mM 2-mercaptoethanol/10 mM Tris·HCl (pH 7.9). Elution was with the same buffer; column fractions (4 ml) were assayed for endonuclease activity as described below using 2 to 4-μl aliquots of column fractions, incubated for 5–16 hr. All fractions containing endonuclease activity were combined, and solid ammonium sulfate was added to 75% saturation. The precipitate was recovered by centrifugation, resuspended in 25–30 ml of DEAE-buffer (10 mM 2-mercaptoethanol/0.1 mM EDTA/10 mM potassium phosphate (pH 7.4)/10% glycerol), and dialyzed against this buffer (three changes of buffer, 2 liters each). The sample was then applied to a column of DEAE-cellulose (Whatman DE 52, 25 × 1.2 cm) that had been equilibrated with DEAE-buffer. The column was washed with 25 ml of DEAE-buffer and was eluted with 200 ml of a linear KCl gradient (0–0.2 M) in DEAE-buffer. If required (see *Results*), fractions of the KCl eluate that contained endonuclease activity were further purified by a second cycle of DEAE-cellulose chromatography.

Enzyme Assays. Assay mixtures for the *Chlamydomonas* endonuclease contained the following in a volume of 0.05 ml: 6 mM $MgCl_2$/6 mM 2-mercaptoethanol/6 mM Tris·HCl (pH 7.9) (standard assay buffer), and 2 μg of DNA substrate. After incubation at 37° for times indicated in the figure legends, the reaction was terminated by the addition of 5 μl of 0.1 M Na_2EDTA (pH 7). Nonradioactive digestion products were analyzed by agarose slab gel electrophoresis (12). The digestion products of ^{32}P-labeled synthetic polynucleotides were analyzed by (*a*) measurement of perchloric acid solubility, (*b*) high-voltage electrophoresis on DEAE-cellulose paper (13), and (*c*) two-dimensional fractionation by electrophoresis on cellulose acetate and homochromatography (14).

Substrates. Adenovirus-2 (Ad-2) DNA was isolated as described (15). Polydeoxyribonucleotides were purchased from

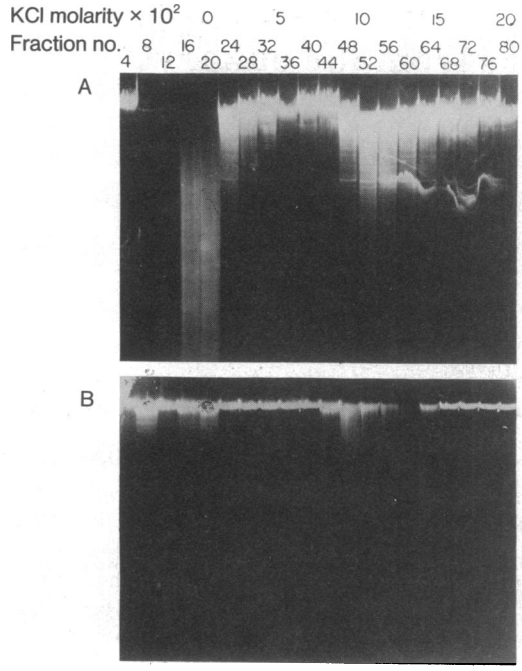

FIG. 1. Assay of DEAE-cellulose column eluates. An aliquot from each of the indicated column fractions was assayed as described in *Materials and Methods*. Fraction numbers are indicated above each gel channel. For both columns, after the sample was loaded, the columns were washed with loading (sample) buffer (fractions 6–20) and the gradient was started while fraction 21 was being collected. (*A*) First DEAE-cellulose column. (*B*) Second DEAE-cellulose column.

PL Biochemicals, Inc., and were labeled *in vitro* by nick-translation (16).

5′-Terminal Nucleotide Analysis of Cleaved Ad-2 DNA. Ad-2 DNA (20 μg) was digested with *Chlamydomonas* endonuclease. Digested DNA was recovered from the reaction mixture either by phenol extraction and ethanol precipitation or after electrophoresis on a 1.4% agarose gel (12) to separate small, single-strand excision products from cleaved and gapped DNA. The digested DNA was then treated with bacterial alkaline phosphatase (obligatory step) prior to labeling the 5′ termini using polynucleotide kinase (17). The 5′-terminal nucleotides were then analyzed after complete digestion with pancreatic deoxyribonuclease and venom phosphodiesterase (18).

Electron Microscopy. Cleavage products of Ad-2 DNA produced by *Chlamydomonas* endonuclease were adjusted to a concentration of 0.5 μg/ml with 10 mM EDTA, mounted on parlodion-coated grids by the formamide technique of Davis *et al.* (19), and examined in a Philips 300 electron microscope. Lengths of cleavage products were measured with a Hewlett-Packard 9864A digitizer from electron micrograph negatives projected by a photographic enlarger.

RESULTS

The *Chlamydomonas* endonuclease was partially purified from crude extracts by gel filtration on Sephadex G-100 followed by one or two cycles of DEAE-cellulose chromatography. An assay of the eluate of the first DEAE- column is shown in Fig. 1*A*, where a specific endonuclease is seen eluting from 0.09 to 0.18 M KCl. This endonuclease is also present in flow-through fractions, where it is contaminated with a nonspecific nuclease. Optimal resolution of the endonuclease bound to the column was obtained only under conditions where it also appeared in the flow-through fractions. Increasing the bed volume of the DEAE-cellulose column so that all of the specific endonuclease was bound resulted in the co-elution of specific and nonspecific nucleases upon application of the KCl gradient. In some enzyme preparations, prolonged incubation of aliquots from fractions eluted by KCl indicated the presence of contaminating exonuclease(s). These preparations were further purified by rechromatography of the initial KCl eluate on DEAE-cellulose. In enzyme preparations that required the additional cycle of DEAE-cellulose chromatography, it was the endonuclease activity in the flow-through, rather than the KCl eluate, that appeared to be exonuclease free (Fig. 1*B*). The purification scheme is summarized in Table 1. The experiments presented here used exonuclease-free fractions from either the first or the second DEAE-cellulose chromatography steps.

Table 1. Purification of *Chlamydomonas* endonuclease

Fraction (ml)	Protein, mg/ml	Endpoint titer*	Specific activity,† units/ mg protein	Total units
Sonication (31)	23.8		—	
High-speed supernate (21)	13.5		—	
Sephadex G-100 eluate (138)	1.51	5-2-6‡	22	6.9×10^3
Eluate of first DEAE-cellulose column (104)	0.075	5-2-6	4.44×10^2	3.47×10^3
Eluate of second DEAE-cellulose column (25.6)	0.0044	5-2-6	7.58×10^3	8.53×10^2

* The sequence of numbers under this heading refers to μl of enzyme, μg of DNA, and hours of incubation, respectively, required to obtain a limit digest (see *Results*).

† With exonuclease-free enzyme fractions, a linear reciprocal relationship was observed between volume of enzyme and length of incubation. The same degree of digestion was obtained when 1 μl of enzyme was incubated for 5 hr or 5 μl of enzyme for 1 hr. One unit of activity: 1 μl of enzyme degrades 2 μg of Ad-2 DNA to a limit digest (see *Results*) in 1 hr at 37°.

‡ In the qualitative agarose gel assay, contaminating exonuclease(s) degrade discrete DNA fragments produced by the endonuclease. Thus, the endpoint titers assigned to eluates from the Sephadex and first DEAE-cellulose columns are approximate.

The pattern of discrete DNA fragments generated by the *Chlamydomonas* endonuclease resembles characteristic patterns obtained after cleaving DNA with a Type II restriction endonuclease (20). However, the patterns differ in that the *Chlamydomonas* enzyme does not produce equimolar yields of fragments and more than 60% of the Ad-2 DNA migrates with an apparent molecular weight between 23×10^6 (intact Ad-2 DNA) and 13.3×10^6 (the *Eco*RI-A fragment of Ad-2 DNA) (21).

Several lines of evidence indicate that this DNA at the top of the gel is refractory to further cleavage. Increasing either the duration of incubation (Fig. 2*A*) or the enzyme concentration (Fig. 2*B*) in a digestion mixture gives no further cleavage of DNA. Furthermore, when cleaved DNA was extracted from a reaction mixture and re-incubated with additional enzyme, no change in the DNA banding pattern or its relative distribution was observed (Fig. 2*C*). These results suggest that the gel fragment pattern observed (e.g., Fig. 2) represents a limit digest.

An aliquot of Ad-2 DNA from a putative limit digest was examined in the electron microscope. The length distribution of molecules measured (Fig. 3) was markedly different from that suggested by the relative mobility of such DNA in an

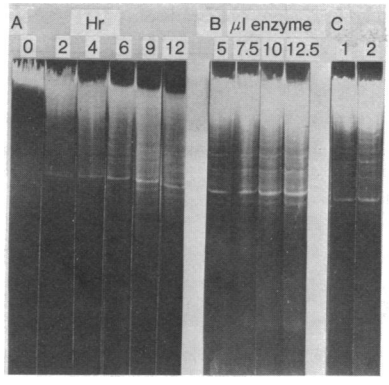

FIG. 2. (*A*) Time course assay of the cleavage of Ad-2 DNA by the *Chlamydomonas* endonuclease. The assay mixture (1.0 ml) contained 40 μg of Ad-2 DNA in standard assay buffer (*Materials and Methods*). The reaction was started by incubation at 37° after addition of a 50-μl aliquot (3.75 μg of protein) of a DEAE-cellulose column eluate with an endpoint titer of 5-2-4. A 50-μl aliquot was taken from the assay mixture at each of the indicated times, mixed with 5 μl of 0.1 M Na_2EDTA (pH 7), and analyzed by gel electrophoresis (12). (*B*) Concentration dependence assay. A DEAE-cellulose (I) fraction with an endpoint titer of 5-2-4 was used. Aliquots of 5 μl (0.375 μg of protein), 7.5 μl, 10 μl, and 12.5 μl were each incubated with 2 μg of Ad-2 DNA for 4 hr at 37° in standard assay buffer and analyzed by gel electrophoresis (12). (*C*) Reincubation of digested Ad-2 DNA with *Chlamydomonas* endonuclease. Ad-2 DNA (10 μg) in 0.25 ml of assay buffer was incubated with 25 μl of enzyme (1.87 μg of protein; titer, 5-2-4) for 4 hr at 37°. The reaction was stopped by addition of 7 μl of 20% sodium dodecyl sulfate. The mixture was adjusted to 0.15 M NaCl and extracted twice with Tris (0.1 M, pH 8)-equilibrated phenol. DNA was recovered by ethanol precipitation, washed with 70% ethanol (−20°) to remove precipitated salts, and then resuspended in assay buffer to a concentration of 40 μg/ml. After reincubation with endonuclease (titer, 5-2-4) aliquots of the reaction mixture were assayed by agarose gel electrophoresis.

agarose gel. Of the 219 molecules measured, only one was larger than the *Eco*RI-A fragment of Ad-2 DNA. The great majority (>70%) of the molecules examined were smaller than 5×10^6 molecular weight.

One striking feature of the DNA as revealed by electron microscopy was the appearance of single-stranded regions within the duplex molecules (Fig. 4, arrows). We have observed

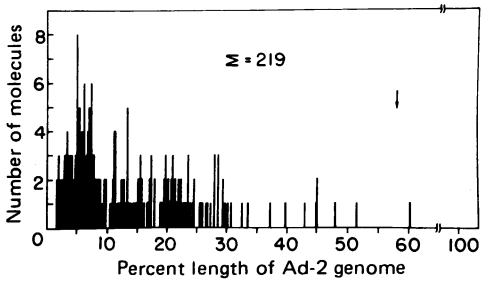

FIG. 3. Length distribution of fragments produced by incubation of Ad-2 DNA with the *Chlamydomonas* endonuclease. A 50-μl sample (2 μg of Ad-2 DNA) was taken from the time course assay (Fig. 2*A*) at the 4-hr time point. The enzyme reaction was stopped by addition of 5 μl of 0.1 M Na_2EDTA (pH 7) in the cold. Ad-2 DNA digested with *Chlamydomonas* endonuclease was mounted for examination in the electron microscope by the formamide basic protein technique of Davis *et al.* (19) with a formamide concentration of 50% in the hyperphase and 20% in the hypophase. The length of the measured molecules is expressed as a percent of the length of intact Ad-2 DNA. The reference length was established by mixing unit length Ad-2 DNA with a digested DNA sample in one of the five spreadings from which the data in Fig. 3 were obtained. Intact DNA was observed only on grids from this spreading. The experimental error obtained for intact Ad-2 DNA was ±2.2% of the unit length. Arrow indicates reference length (57.8%) of *Eco*RI fragment A of Ad-2 DNA (21).

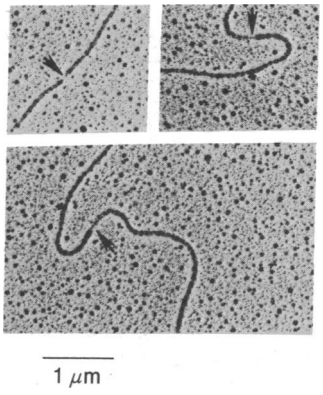

FIG. 4. Demonstration of single-strand regions in fragments of Ad-2 DNA after incubation with *Chlamydomonas* endonuclease. Ad-2 DNA from the 4-hr point of the time course assay (Fig. 2) was prepared as described in the legend of Fig. 3. Arrows point to single-strand regions.

gaps as long as 250 ± 50 nucleotides. When an aliquot of digested DNA at high concentration was prepared for electron microscopy, a significant number of branched and anastomosing molecules were seen; no such structures were observed in undigested DNA spread at the same concentration.

The disparate size estimates obtained from gels and from electron micrographs can be reconciled if molecular interactions between these gapped molecules lead to retarded mobility in the agarose gel. To examine this possibility, we incubated predigested, gapped Ad-2 DNA with the single-strand specific endonuclease S_1 of *Aspergillus oryzae* (22). The results (Fig. 5) show that after S_1 digestion, the large amount of DNA usually seen at the top of the gel after incubation with the *Chlamydomonas* endonuclease is no longer present, suggesting that the single-strand gaps are responsible for the anomalous migration of the cleaved DNA in the agarose gel.

The specificity of cleavage of the *Chlamydomonas* endo-

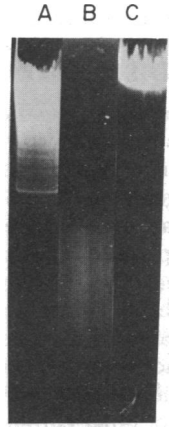

FIG. 5. Susceptibility of Ad-2 DNA fragments produced by *Chlamydomonas* endonuclease to S_1 nuclease. A 0.10-ml mixture of 4 μg of Ad-2 DNA in standard assay buffer was incubated with a 10-μl aliquot of enzyme (0.75 μg of protein; titer, 5-2-4) at 37° for 4 hr. DNA in the reaction mixture was recovered by phenol extraction and ethanol precipitation. The precipitated DNA was resuspended in 0.10 ml of 4.5 mM $ZnSO_4$/325 mM NaCl/30 mM acetate (pH 4.4) (S_1 nuclease buffer). (*A*) Gel electrophoresis of a 50-μl sample of resuspended DNA prior to digestion with S_1 nuclease. (*B*). Gel electrophoresis of resuspended DNA after incubation with 40 units of S_1 nuclease (the generous gift of A. Efstradiatis) at 25° for 30 min. (One unit will cause 1.0 μg of nucleic acid to become perchloric acid-soluble per min at pH 4.6 at 37°.) (*C*) Gel electrophoresis of 2 μg of Ad-2 DNA after incubation with 40 units of S_1 nuclease in S_1 nuclease buffer at 25° for 30 min.

Table 2. 5′-Terminal nucleotide analysis of cleaved and gapped Ad-2 DNA*

5′-Terminal nucleotide	%
T	66
G	26
A	5
C	3

* Procedures used to determine 5′-terminal nucleotides are described in *Materials and Methods*. Values presented are the average of three experiments (±5%, T and G; ±1%, A and C). Two analyses were performed on fragments directly isolated from the enzyme reaction mixture; one assay used fragments recovered from an agarose gel. A typical labeling experiment used 0.13 pmol of Ad-2 DNA. Incorporation prior to digestion with *Chlamydomonas* endonuclease was $<10^{-4}$ pmol; after endonuclease digestion it was 0.68 pmol. Efficiency of the kinase reaction is not known; however, parallel experiments with restriction endonuclease fragments bearing a 5′-terminal tetranucleotide extension gave an efficiency of 32% of the theoretical incorporation.

nuclease was examined by analysis of the 5′-terminal nucleotides present after Ad-2 DNA is cleaved (Table 2). Thymidylic acid is the major 5′-terminal nucleotide, with deoxyguanylic acid also present in abundance. The other two nucleotides account for only 10% of all 5′ termini.

These findings prompted us to examine the cleavage of single- and double-stranded synthetic polynucleotides of defined sequence (Table 3). Surprisingly, cleavage only occurred in polymers containing thymidylic acid. The alternating copolymer poly[d(G-C)] was not a substrate for the enzyme, nor was poly(dG)·poly(dC). The finding that poly[d(A-T)]·poly[d(A-T)] was degraded to dT-A and oligomers of this dinucleotide, while poly(dA)·poly(dT) gave monomer and oligomers of T confirms that the initial endonucleolytic scission takes place at the phosphodiester bond 5′ to thymidylic acid but not at the bond 5′ to deoxyadenylic acid. These data also indicate that, among susceptible substrates, there is a clear preference by the enzyme for the duplex configuration.

DISCUSSION

This paper describes the partial purification and preliminary characterization of a novel endodeoxyribonuclease. The enzyme appears to act endonucleolytically at specific sites on homoduplex DNA to generate double-strand fragments containing single-strand gaps. We believe the formation of these gaps is an inherent property of the enzyme rather than due to a contaminating enzyme (either specific or nonspecific) for the following reasons: (*i*) The characteristic electrophoretic pattern of digested Ad-2 DNA appears to be a consequence of both the cleaving and gapping activity(ies) we attribute to the enzyme. In the course of the purification scheme described here and also during a wide variety of other chromatographic procedures, including chromatography on phosphocellulose, hydroxyapatite, single- and double-stranded DNA-agarose and several ω-amino-alkyl Sepharoses (data not shown), we have yet to observe a change in the characteristic electrophoretic pattern of digested Ad-2 DNA. This suggests that throughout these purification regimes we have been unable to separate or change the ratio of the cleaving and gapping activities. (*ii*) The activity of the enzyme on synthetic polynucleotides is highly specific. If contaminating enzymes had been present, we would not have observed either the specificity of attack on poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT), or the absence of cleavage of the other polynucleotides tested, i.e., poly[d(G-C)]·poly[d(G-C)] and poly(dG)·poly(dC) (Table 3). (*iii*) The pattern

Table 3. Analysis of cleavage products of ^{32}P-labeled synthetic polynucleotides

Substrate*	Form†	% acid-soluble counts‡	Products	%
Poly[d(**A**-T)]	ds	65	pT-A	42
			pT-A-T-A	45
			pT-A-T-A-T-A	13
	ss	4	N.A.	
Poly[d(A-**T**)]	ds	41	pT-A	43
			pT-A-T-A	42
			pT-A-T-A-T-A	16
	ss	3	N.A.	
Poly(d**A**)·poly(dT)	ds	0.1	nil	
	ss	0.2	nil	
Poly(dA)·poly(d**T**)	ds	44	pT	18
			pT-T	15
			pT-T-T	17
			pT-T-T-T	19
			pT-T-T-T-T and longer	31
	ss	6	N.A.	
Poly[d(**G**-C)]	ds	1	N.A.	
	ss	3	N.A.	
Poly[d(G-**C**)]	ds	2	N.A.	
	ss	6	N.A.	
Poly(d**G**)·poly(dC)	ds	4	N.A.	
Poly(dG)·poly(d**C**)	ds	3	N.A.	

N.A. = not assayed.

* **Boldface** type indicates [α-^{32}P]nucleoside triphosphate used for *in vitro* labeling of synthetic polynucleotides.

† ds = double-strand configuration; ss = single-strand configuration.

‡ Reaction mixtures containing 2 μg of substrate (1.5×10^5 cpm), with and without enzyme, were incubated at 37° for 4 hr. Reactions were terminated by cooling and addition of two volumes of cold 0.35 M perchloric acid. After 30 min at 0°, reaction mixtures were centrifuged (12,000 × *g*, 15 min). Radioactivity in the supernatant fraction was then measured in a liquid scintillation counter. Percent acid-soluble counts were determined from the difference in cpm between reaction mixtures ± enzyme.

of double-stranded cleavage of Ad-2 DNA, which has facilitated the assay of this endonuclease, is unchanged upon prolonged incubation with enzyme (Fig. 2*A*) subsequent to reincubation (Fig. 2*B*) or in the presence of excess enzyme (Fig. 2*C*).

The pattern of double-strand cleavages of Ad-2 DNA has provided a convenient means to assay the enzyme during purification. However, it is our view that the discrete array of double-strand breaks produced in Ad-2 DNA is not due to the concerted scission of duplex molecules. Rather, we propose that these cleavages are a consequence of the production of overlapping gaps on complementary strands of DNA. The limited number of these overlap regions in DNA molecules such as Ad-2 argues for an enzyme with a considerable degree of specificity. The failure to detect discrete fragments subsequent to endonuclease S_1 digestion of cleaved and gapped Ad-2 DNA suggests the frequency of single-strand cleavages may be significantly greater than that inferred from the limited number of double-strand scissions. However, the absence of bands may be due to inadequate resolving power of the 1.4% agarose gel.

Gap formation may occur when several cleavage sites occur in close proximity on one strand of DNA; the oligonucleotides between two adjacent nicks may then melt off the DNA to produce a single-strand gap. However, this mechanism does

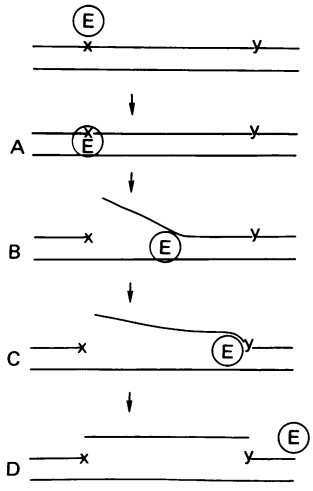

FIG. 6. Model for the action of the *Chlamydomonas* endonuclease. x = site of initial endonucleolytic cleavage (incision site). y = site of second cleavage (excision site). E = enzyme. Details of the sequence of events illustrated are presented in the *text*.

not appear to account for all gap formation since some gaps observed in Ad-2 DNA are of considerable length (i.e., 250 ± 50 nucleotides).

A diagram that illustrates our current working hypothesis of the mode of action of this endonuclease is shown in Fig. 6. In this scheme, the enzyme makes an initial endonucleolytic, single-strand cleavage (*incision*) at x (Fig. 6*A*). The enzyme then moves along the DNA molecule displacing oligonucleotides (Fig. 6*B*) until it reaches another recognition site, y, where a second single-strand cleavage (*excision*) occurs (Fig. 6*C*), producing a gap in duplex DNA and an excised oligonucleotide(s) (Fig. 6*D*). Although Fig. 6 implies that the excised sequence of oligonucleotides is intact after displacement, we do not have unequivocal evidence for this point.

One appealing aspect of this model is that it resolves the apparent disparity between the two analyses of cleavage specificity. Analysis of the 5′-terminal nucleotides of digested Ad-2 DNA (Table 2) showed about 30% G, in marked contrast to the results obtained with homopolymers and alternating copolymers containing G (Table 3). These latter data indicate that little or no cleavage occurs in substrates containing only G, C, or G-C residues. If the incision site x requires a T whereas the excision site y can contain either a T or a G residue 5′ to the enzymatic cut, then both nucleotides would be seen as 5′ termini in cleaved and gapped Ad-2 DNA, as we find. However, because the synthetic polynucleotides containing only G, C, or G-C used in our experiments do not contain *incision* sites (i.e., T) recognized by the enzyme, no cleavage can occur. Thus, we conclude that certain other sequences, containing G, but not present in the synthetic polynucleotide used here, are also substrates for this enzyme. Clearly, further studies of both natural DNAs and more sophisticated synthetic DNAs will be required to identify the specific sequences recognized by this enzyme.

Single-strand breaks and discontinuities appear to be a common feature in the major pathway of genetic recombination in a wide range of organisms. In their general model for recombination and gene conversion, Meselson and Radding (23) propose that a single-strand break in one molecule of two homologous DNA duplexes aligned during meiosis serves as a site of enzymatic strand displacement, leading to symmetric or assymetric strand transfer. These modes of molecular exchange have been proposed to account for reciprocal exchanges, i.e., recombination, and for nonreciprocal events, seen as aberrant allelic segregation ratios, and referred to as gene conversion.

Recently, Hotta and Stern (24) have shown that in meiotic *Lilium* microsporocytes, single-strand gaps appear in a specific set of unique sequences of zygotene chromosomes. However, these gaps do not seem to play a role in recombination.

In *Chlamydomonas*, chloroplast genes undergo both reciprocal and nonreciprocal exchange events in vegetative cells. Nonreciprocal events occur with the unprecedented frequency of 40% per doubling for each locus that has been examined (8, 9, 25). Preliminary results suggest that the enzyme described here is localized in the chloroplast (unpublished observations).

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