Release of High Molecular Weight DNA from *Neurospora crassa* using Enzymic Digestions

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Methods are described that allow extraction of high molecular weight DNA from germinated conidia of *Neurospora crassa*. By labelling DNA with ribonucleosides, early conidia were shown to be active in DNA synthesis. These cells when treated with the enzyme Zymolyase became fragile and could be readily lysed with ionic detergents to release high molecular weight DNA. The DNA extracted from Zymolyase treated cells on to alkaline sucrose gradients sedimented as a heterogeneous species of up to \(150 \times 10^6\) molecular weight. A minor DNA species (presumably mitochondrial) of \(20 \times 10^6\) molecular weight comprised 2-7% of the total. The identity of the DNA was confirmed by sensitivity to DNAase, the diphenylamine assay and TLC. Sedimentation patterns were unaffected by protease digestions and no anomalous high speed rotor effects were evident. Isopycnic gradients suggested that the DNA released was uncomplexed with either protein or carbohydrates. Sepharose chromatography of extracted, RNAase-treated Zymolyase lysate resulted in clearly separate high molecular weight DNA and RNA-protein elution profiles.

UV light preferentially inhibited nuclear DNA synthesis and drastically reduced the size and amount of nascent DNA being synthesized in the excision defective *uss-2* mutant. Sites in parental DNA sensitive to *Micrococcus luteus* UV endonuclease were measured in cells made permeable with Triton X-100.

INTRODUCTION

Biochemical studies of *Neurospora crassa* DNA repair have been hampered by the inability to obtain specific isotopic labelling of DNA (Grivell & Jackson, 1968), by difficulty in sampling small portions of the filamentous fungus, and by the refractivity of the cell wall to gentle lysis. We plan to devise methods to overcome these difficulties and to determine that high molecular weight DNA released from the cell by ionic detergents was uncomplexed with other cell components. We have also initiated studies of DNA repair processes in *N. crassa*.

The enzyme Zymolyase provides an alternative to the snail gut preparations that have been used in the past to remove the wild-type cell walls from *N. crassa* (Worthy & Epler, 1972). The snail enzyme mixtures are often contaminated with nucleases and cell wall hydrolysing activities are very variable (our laboratory). Several workers have chosen to work with cell wall-less mutants of *N. crassa*. Unfortunately, simple genetic manipulations are severely limited in these slow growing slime strains (Emerson, 1963). Use of Zymolyase, will permit the numerous cell wall-containing *N. crassa* DNA repair mutants (Schroeder, 1975) to be used in the elucidation of the molecular biochemistry of fungal nucleic acids.
METHODS

Materials. The [5,6-\textsuperscript{3}H]uridine and [2,8-\textsuperscript{3}H]adenosine each of specific activity 40 Ci mmol\textsuperscript{-1} (1.48 TBq mmol\textsuperscript{-1}) and [2-\textsuperscript{14}C]uridine of specific activity 52.4 mCi mmol\textsuperscript{-1} (1.94 GBq mmol\textsuperscript{-1}) were purchased from ICN, Irvine, California. Chemicals were of reagent grade and purchased from various commercial sources, principally from Sigma. RNAase-free sucrose for centrifugation was purchased from Bio-Rad. Zymolyase 60000 was purchased from the Kirin Brewing Company, Miyahara, Takasaki 370-12, Japan.

Deionized, glass distilled, autoclaved water was employed for all aqueous solutions. To prevent exogenous nuclease contamination, all glassware used was washed in detergent containing diethyl pyrocarbonate, and oven baked at 150 °C for at least 48 h.

Cell material. All work was done either with the wild-type strain, 74-OR23-1A, or the excision-repair defective mutant, uvs-2 (S-633), of Neurospora crassa. This mutant strain is a sixth generation backcross of the original uvs-2 mutant (Stadler & Smith, 1968) to the standard N. crassa wild-type strains 74-OR23-1A and 74-OR8-1a. Methods for maintaining and growing cultures are given in Schroeder (1970a,b). Conidia were harvested and filtered through eight layers of sterile cheesecloth and added to growth media (Vogel, 1964) and incubated at 30 °C in a water bath rotary shaker.

Microscopic examination of conidia. Unstained conidia were examined at a magnification of 450(X) with a Zeiss binocular microscope with a built-in light source.

DNA labelling. The method of Worthy & Epler (1972) was used throughout this work, except that tritiated adenosine in addition to tritiated uridine was used for labelling cellular nucleic acids. In some experiments [\textsuperscript{14}C]uridine was used to doubly label the DNA. Final radioisotope concentration in the growth media ranged from 0.5 μCi ml\textsuperscript{-1} to 25 μCi ml\textsuperscript{-1}, depending on the experiment.

Zymolyase digestion. The method was essentially that of Cryer et al. (1975), except that Zymolyase 60000 was used. Only the 1 M-sorbitol solubilized protein of the Zymolyase preparation was used to digest the cells. Typically, 200 μg of the solubilized protein (as determined by the Lowry method) per 250 × 10\textsuperscript{6} cells in 2-5 ml buffer were used. Generally, conidia germinated for 2 to 3 h were used in the DNA extraction experiments. The digestion was at 30 °C for 10 to 15 min unless indicated otherwise.

DNA extraction from zymolyase digested cells. Digested cells were rinsed twice in 1-0 M-sorbitol, 0-1 M-sodium citrate pH 7-5 and 60 mM-EDTA (Cryer et al., 1975) and collected by centrifugation. The final pellet was lysed in 1% SDS or 1% Sarkosyl (U.S. Biochemicals, Cleveland, Ohio) containing 50 mM-Tris pH 8-0 and 10 mM-EDTA. Protein was extracted with phenol-chloroform (Reynolds, 1978). Dialysis was done using cellulose dialysis bags generally against 3000 volumes of 0.1(X) standard saline citrate; 1(X) standard saline citrate is composed of 0-15 M-NaCl and 0-015 M-sodium citrate. Sepharose-4B chromatography was done as described by Lurquin et al. (1975) and the diphenylamine assay of DNA as described by Richards (1974), with calf thymus DNA as a standard.

Determining radioactivity in acid-precipitable materials. Calf thymus DNA (25 μg) was added to samples before the addition of cold (0-4 °C) 10% (w/v) trichloroacetic acid (TCA) containing 50 μg uridine and adenosine ml\textsuperscript{-1}. The precipitated samples were washed on to presoaked GF/F glass filter circles (Whatman). The filters were rinsed and counted by liquid scintillation as described by Rupp & Howard-Flanders (1968). In some experiments, samples were added and counted directly in liquid counting scintillation fluid (ACS-Amersham, U.K.) containing small volumes of water. Counting efficiencies ranges from 25 to 35% for \textsuperscript{3}H and 50 to 60% for \textsuperscript{14}C.

 Determination of the identity of TCA-precipitable counts. TLC was performed as by Carrier & Setlow (1971). Spots were visualized using UV fluorescence, or the plates were cut into strips and counted in an aqueous scintillant. Spots were identified by comparison with the R<sub>e</sub> values of known bases (Mangold, 1969).

Enzyme digestions were done by placing radioactive samples in their appropriate buffer (as suggested by the supplier) with either DNAase I (RNAase-free, Worthington Biochemical Corp.) (Price, 1975), RNAase I or proteinase K (Miles Laboratory, Elkhart, Indiana). Enzyme concentrations ranged from 20 to 200 μg ml\textsuperscript{-1}. The RNAase was dissolved in water and boiled for 5 min before use. The proteinase K was self-digested for 30 min at 39 °C before use. Acid-insoluble counts were determined as described above.

Alkaline sucrose density sedimentation gradients. For alkaline sucrose gradient sedimentations the methods of Studier (1965) and Martin & Ames (1961) were combined. Cells were lysed in a 150 μl layer of 0-5 M-NaOH, 3% (w/v) Sarkosyl, 2% (w/v) SDS (Bio-Rad), 1% (w/v) sodium-deoxycholate and 20 mM-EDTA on top of the gradient and gently stirred with a pin to release the DNA. Centrifugation was at 40000 r.p.m., unless otherwise stated in a SW 50.1 rotor (Beckman) at 22 °C, for 70 to 150 min, depending on the need. Fractions were collected by pumping from the bottom of the polyallomer tubes using a National peristaltic pump. To hydrolyse the RNA further so that it would not precipitate in TCA, incubation of fractions was continued at 37 °C for 18 h.

Molecular weight DNA markers. The tritiated SV40 covalently closed DNA (Form I) was kindly provided by Dr Ben Archer (University of Idaho, Moscow, Idaho). This marker has an S value of 53 on alkaline sucrose and an apparent molecular weight of 32 × 10\textsuperscript{6}.
Enzymic release of DNA from \textit{N. crassa}

\textit{Molecular weight calculations.} The molecular weight of peak fractions in the sedimentation velocity experiments were calculated using the formulae of Studier (1965) and Martin & Ames (1961).

\textit{UV treatment.} Methods for administration of UV are given in Schroeder (1970a,b).

\textit{Micrococcus luteus dimer endonuclease assay in permeabilized cells.} The UV dimer-specific endonuclease was purified and stored as described by Reynolds (1978). The enzyme extract (0.05 to 0.1 vol.) was added to conidia germinated for 2 to 3 h which had been digested with Zymolyase, and in a final concentration of 0.2\% (v/v) Triton X-100 and incubated at 37 °C for 30 min. Cells were lysed, DNA sedimented and acid-insoluble radioactivity measured as described above.

\textit{CsCl isopycnic gradient centrifugations.} Extracted, dialysed DNA was added to dry CsCl and the final density adjusted to 1.725 at 22 °C by using an Abbe temperature controlled refractometer. Centrifugation was at 38000 r.p.m. for the first 18 h and then at 33000 r.p.m. for an additional 46 h in a SW 50.1 rotor. Tubes (nitrocellulose, Beckman) were punctured and the fractions collected from the bottom. Samples were brought to 0.5 M with NaOH and placed at 37 °C for 18 h before measurement of acid-insoluble radioactivity.

\textbf{RESULTS}

\textit{Conidial germination and DNA incorporation of radioactivity}

Cultures of germinating conidia were used throughout this project because accurate sampling of the entangled mass of hyphae in mycelial cultures was not feasible. Very early conidia could be pipetted and centrifuged much like other non-filamentous micro-organisms. Ungerminated conidia (4 to 6 d old) in minimal media took up to 4-5 h to achieve 90\% germination. It was also clearly evident that DNA synthesis commences during early germination events (within 20 min). The synthesis rate was found to be linear for at least 5 h after inoculation into growth media (data not shown).

\textit{Zymolyase digestion of germinated conidia}

The degree of cell wall digestion was monitored by determining the susceptibility of the sample to lysis when Sarkosyl was added. If successful lysis occurred the cell suspension of 50 x 10\(^6\) conidia in 1 ml buffer became markedly viscous. Unlike with other cell wall hydrolysing enzyme preparations used for \textit{N. crassa} (Worthy & Epler, 1972; Agsteribbe, 1979), the cells here never formed spherical protoplasts even after several hours of digestion under optimum conditions. After Zymolyase digestion, the cells appeared microscopically indistinguishable from undigested cells and consequently this criterion could not be used to monitor cell wall removal.

The digestion did not proceed as completely at 2 °C as at 30 °C. There was no advantage in increasing the temperature of incubation to 37 °C. \(\beta\)-Mercaptoethanol seemed slightly, SDS markedly to inhibit the digestion, whereas the enzyme appears to be completely active in the presence of Triton X-100. SDS or Sarkosyl was equally effective in lysing Zymolyase-digested cells but these detergents had no effect on cells not treated with Zymolyase. Identical results were obtained using wild-type or excision-repair defective cells. The Zymolyase digestion proceeded equally well in citrate or Tris buffers, with either mannitol or sorbose as osmotic support.

Zymolyase had no significant activity on ungerminated conidia. The conidial germ tube must be visible for efficient digestion. Cell survival after Zymolyase digestion was typically 41–49\% of control (undigested) cells. Protein from dissolved Zymolyase (200 \(\mu\)g) was adequate to digest up to 7.5 mg dry weight of conidia germinated for 3–6 h. However, the enzyme was much less effective with conidia germinated for more than 6 h. This may be because the germ tube lays down very thick walls at longer times (Farkas, 1979). Also, the addition of chitin-like structures to mycelium may make cell walls refractory to digestion.

The Zymolyase preparation was stable for at least 3 years stored desiccated at 4 °C but the activity diminished rapidly in solution, perhaps because of contaminating proteases. We could not detect any nuclease activity in the enzyme. The preparation gave no DNA hyperchromicity increases with purified calf thymus DNA (Sigma) nor did it reduce the amount of acid-insoluble counts recovered on filters.
Radioactively labelled ([\textsuperscript{3}H]uridine and [\textsuperscript{3}H]adenosine), Zymolyase digested cells released high molecular weight DNA when confronted with a low osmotic strength buffer, EDTA, and ionic detergents (Fig. 1). This rapidly sedimenting, alkali-stable, acid-insoluble material was DNA. Diphenylamine assays done on similar gradients confirmed the identity of the peak fractions. Typically the amount of DNA in fraction 3 was 0.34 µg, in 17 was 0.10 µg and in 15 was 0.14 µg. Also, the peak of radioactivity around fraction 3 was very sensitive to DNAase. Thus in gradients performed before and after DNAase treatment a sample from fraction 3 contained 2495 and 285 counts, respectively. A formic acid hydrolysate of the peak fractions in this experiment, chromatographed on cellulose thin layer plates, confirmed the presence of tritiated thymine, cytosine and adenine, representing 20%, 45% and 35%, respectively, of the total radioactivity. No significant counts above background were obtained in the uridine region of the thin layer plate, thus confirming that removal of the RNA by alkaline hydrolysis was complete. The molecular weight of the fast sedimenting DNA species in Fig. 1 was approximately 150–160 × 10\(^6\). This is a typical sedimentation pattern for DNA released on top of alkaline gradients (Boyd & Setlow, 1976; Regan & Setlow, 1974; Di Caprio & Cox, 1981). Varying the DNA concentration between 0.1 and 7 µg per gradient produced no aggregation effects. A minor distinct DNA species was also generally evident within the alkaline sucrose gradient at or near fraction 15, 0.30 of the tube length from the meniscus (Fig. 1). This minor peak represented between 2–7% of the total radioactivity and was most likely mitochondrial DNA. It sedimented with an apparent molecular weight of 20 × 10\(^6\), as found for \textit{N. crassa} mitochondrial DNA (Terpstra et al., 1976). The true identity of this minor peak might be established by restriction analysis since the map is known for \textit{N. crassa} mitochondrial DNA (Terpstra et al., 1976). The radioactive material remaining at the top of the gradient was degraded fragments of DNA.

The DNA sedimentation profile was unchanged by proteinase K digestion before centrifugation. Therefore, the possibility of gross protein–DNA complexes producing anomalous sedimentation patterns is unlikely. The denaturing conditions of the isokinetic gradients were expected to disrupt any possible chromatin-like structures existing in the cell.
Enzymic release of DNA from N. crassa

To test for possible anomalous rotor effects of high speed centrifugation on the DNA sedimentation profile, centrifugations were also done at reduced speed (9000 r.p.m.) for extended periods of time (21 h) after overnight lysis before centrifugation. No anomalous pattern of DNA sedimentation was evident. Similar DNA molecular weight values for marker (SV40), nuclear and for minor species (presumably mitochondrial DNA) were obtained (data not shown).

Sepharose-4B chromatography of Zymolyase lysates

In order to provide a rapid (2–3 h) isolation of native DNA free from RNA or protein contamination, we decided to determine the usefulness of molecular exclusion column chromatography of Zymolyase–SDS lysates (Lurquin et al., 1975). When crude lysates were used both DNA and RNA were excluded with protein in the void volume of the column. Gentle phenol-chloroform protein extraction or exhaustive proteinase K digestion of the lysates did not improve the separations. However, when the extracted sample was dialysed against 1(X) standard saline citrate buffer and briefly digested with small amounts of RNAase (50 µg ml\(^{-1}\), 30 min), the separation of DNA from RNA and protein was greatly improved (Fig. 2). The DNA was found in the excluded (void volume) peak (peak A) and was clearly separated from the partially retarded RNA and protein peak (peak B). Peak A material showed only a sensitivity to DNAase (100% hydrolysed). Digestion with proteinase K and RNAase did not reduce the amounts of acid-insoluble material in the sample. Consequently, the material in peak A was DNA. The molecular weight of the DNA in peak A is shown in the inset to Fig. 2. This DNA had a single-stranded molecular weight of \(8.7 \times 10^6\) on sucrose alkaline gradients, was fairly homogeneous in size and reacted quantitatively with diphenylamine reagent. The 260/280 nm absorbance ratio was 2.35, indicating no significant amount of protein contamination, and the absorbance maximum was at 257 nm. The specific radioactivity of this DNA was 28,300 d.p.m. µg\(^{-1}\).
The material in peak B showed sensitivity to RNAse (~97% hydrolysed) and was completely hydrolysed with NaOH, yet 12% of the material was also made acid-soluble with proteinase K digestion. The 260/280 nm absorbance ratio was 1.65-1.70, strongly suggesting protein contamination. The specific radioactivity of the component of peak B that was sensitive to RNAse, based on its absorbance at 260 nm was 91600 d.p.m. per µg RNA. This was likely to be an underestimate due to the protein contamination and its effect on the 260 nm reading. The highest S value of this radioactive material as determined on neutral sucrose gradients was less than 21S.

On the basis of these estimates, the total nucleic acids obtained in this experiment (Fig. 2) consisted of 90% RNA and 10% DNA by weight. These values are in good agreement with what was found earlier in *N. crassa* (Worthy & Epler, 1972). Due to differences in specific activity of labelling, labelled DNA represents approximately 3-3% of the total radioactivity in the sample. Total DNA yield relative theoretical values (Horowitz & Macleod, 1960; Minagawa et al., 1959) was, within experimental error, 100%.

**Buoyant density gradients of UV-irradiated pulse-labelled DNA**

A dose of UV light, 40 J m\(^{-2}\), was found to kill all but 3 to 4% of the conidia from the *wvs-2* strain, but had no effect on survival of the wild-type strain. The rate of incorporation of counts into DNA was also reduced to approximately 21% of control. When these cells were pulse-labelled for 45 min after such a UV dose and their DNA was extracted and banded in CsCl, the pattern of counts was markedly altered from that of control unirradiated pulse-labelled cells. The control DNA banded at density 1.714 g cm\(^{-2}\) with a minor species forming a reproducible shoulder at 1.701 to 1.699 g cm\(^{-2}\) (Fig. 3). This shoulder probably represented mitochondrial DNA and accounted for approximately 5 to 10% of the total counts in the gradient. These density values are in close agreement with earlier reported values for *N. crassa* nuclear DNA (Dutta et al., 1967; Schildkraut et al., 1962) and for *N. crassa* mitochondrial DNA (Luck & Reich, 1964; Villa & Storck, 1968). Similar profiles were obtained in the CsCl gradients whether or not the DNA released from Zymolyase digested cells was phenol-chloroform extracted or unextracted. If any covalent protein or carbohydrate complex was to form with the DNA it should persist through the procedure and change (probably decrease) the buoyant density of the DNA.

In the UV-irradiated sample, although peak densities remained the same, the proportion of counts in each DNA band was markedly changed toward a greatly enriched mitochondrial fraction (Fig. 3). Even though the nuclear DNA fraction still represented the majority (about 62%) of the total, counts in the mitochondrial fractions now represented approximately 38% of the total. Certainly, after UV irradiation, mitochondrial DNA shows a much enriched (relative to nuclear DNA) replication activity. These percentage calculations are based on the considerations determined significant or important by Lurquin et al. (1972). Sample and centrifugation conditions were set up nearly ideally for maximum resolution of these DNA preparations using the SW 50.1 rotor. The gradients contained less than 1 µg DNA and the DNA was mechanically sheared to 8–9 × 10\(^6\) molecular weight before the centrifugation. It was therefore expected that the separation and resolution obtained were in fact valid.

**UV dimer endonuclease assay in Triton X-100 permeabilized cells**

After UV irradiation, the presence of dimers in the DNA and a cell's repair response may be recorded by several methods, including assay for pyrimidine dimer-specific endonuclease sensitive sites (Wilkin, 1973). By treating UV-irradiated, Zymolyase digested cells with Triton X-100 it was possible to make these cells permeable to *M. luteus* UV dimer endonuclease. Similar permeability to endonuclease has been reported earlier in spherooplasted yeast cells (Di Caprio & Cox, 1981; Resnick et al., 1981). When high molecular weight [\(^{14}\)C]DNA was treated with endonuclease, the control DNA sedimented as a 150 × 10\(^6\) molecular weight species, whereas after endonuclease treatment the DNA was depolymerized to approximately 50 to 60 × 10\(^6\) molecular weight (Fig. 4). This gave a rough estimate of interdimer sites within the DNA.
Enzymic release of DNA from N. crassa

Fig. 3. CsCl isopycnic gradients of UV-irradiated, pulse-labelled DNA. Density (×) increases from right to left. Density (refractive index, ηb) was measured with a temperature controlled ABBE refractometer. All counts are alkali-stable and acid-insoluble. ○, Control cells; ●, UV-irradiated cells. Each gradient contained at least 26000 c.p.m. Density determinations were made at the cross check marks indicated.

The effect of UV on the molecular weight of nascent DNA being synthesized during a 45 min pulse in the uvs-2 strain is also shown in Fig. 4. Using a double radioactive labelling scheme it was possible to distinguish newly made DNA (nascent) from parental (template) DNA. Cellular DNA was labelled for 2 h during germination with [14C]uridine. Cells were then placed in fresh media without isotope and grown for another 1.25 h. At which time the cells were exposed to UV and finally pulsed for 45 min with [3H]uridine. The tritium label (nascent-3H) run in the same gradient as the control (template-14C) showed that only low molecular weight DNA was being synthesized. The molecular weight of this broad peak of nascent DNA ranged from 10 to 30 \times 10^6. In these irradiated cells there was also a great deal of fragmented DNA located at the top of the gradient. During a 45 min pulse, unirradiated cells from the uvs-2 strain synthesized DNA up to 150 \times 10^6 molecular weight (result not shown). Experiments are in progress to determine if this low molecular weight DNA synthesized after UV irradiation was later converted to high molecular weight DNA more like the control sample.
DISCUSSION

For many studies of nucleic acid transactions, including DNA repair and replication in *N. crassa* germinating conidia must be used because only during this life stage can small portions be sampled, uniform chemical and radiation doses given, and serial dilution plating used to measure survival.

Indirect (ribonucleosides) DNA labelling methods, followed by removal of RNA, have been shown by Worthy & Epler (1972) to be useful in labelling *N. crassa* DNA. DNA was labelled using ribonucleosides followed by hydrolysis of RNA because *N. crassa* has no thymidine kinase (Grivell & Jackson, 1968). By including triitated adenosine with uridine we have achieved rapid labelling of DNA to moderate levels of specific activity. Very early conidia (2 to 3 h) were found to be active in DNA replication.

The problem of consistently extracting uncomplexed, high molecular weight DNA from germinating conidia on to isokinetic velocity gradients has been solved by using a commercially available, partially purified lysing enzyme, Zymolyase 60 000. Cells briefly treated with this enzyme readily lye in ionic detergents. Unlike what has been seen in some mammalian DNA extraction studies (Hatayama & Goldberg, 1979; Elkind & Kamper, 1970) the released DNA was not complexed with any detectable non-DNA cell component as determined by isopycnic density centrifugations or proteinase treatments before velocity centrifugations. In one report carbohydrate was found to co-purify with mycobacterium DNA (Hill et al., 1972). The high molecular weight DNA also does not suffer any serious rotor effects during high speed centrifugation as has been seen in another eukaryote (Dingman & Kakunaga, 1976).

The inhibitory effect of UV irradiation on DNA replication has also been demonstrated here using Zymolyase released DNA and both isopycnic and alkaline sucrose gradient centrifugation. A low dose (40 J m⁻²) reduces replication in the *us-2* strain by 78% and greatly reduces the molecular weight of nascent DNA synthesized during a 45 min pulse. Control cells could synthesize DNA with up to five times higher single-stranded molecular weight (during a 45 min pulse) than irradiated cells. A similar finding has been shown in yeast by Di Caprio & Cox (1981). Preferential inhibition of nuclear DNA replication compared to mitochondrial DNA replication was also seen in *N. crassa*. Kielman & Deering (1980) have recorded similar findings in Dictyostelium discoideum (slime moulds). The reason for this may be reduced target size of mitochondrial DNA which therefore might suffer fewer UV hits (Sancar et al., 1979).

The experiments with Triton X-100 permeabilized cells and *M. luteus* UV dimer endonuclease demonstrated that this assay can be used effectively in Zymolyase digested, UV-irradiated *N. crassa* cells. However, if the finding of DNA synthesis blocks at each UV-induced cyclobutane dimer in *Escherichia coli* (Rupp & Howard-Flanders, 1968) holds true for *N. crassa*, then the endonuclease did not cleave the DNA at every dimer site, since the interruption of DNA synthesis produced lower molecular weight fragments than the endonuclease assay predicted for interdimer distances. On the assumption that UV dimer induction in the DNA was random, the endonuclease was cleaving at only 15% of the dimer sites under the conditions in these experiments. This similar, incomplete digestion has been seen under certain conditions in permeabilized human cells (Zeeland et al., 1981), in Triton X-100 permeabilized yeast cells (Di Caprio & Cox, 1981; Resnick et al., 1981), and in *E. coli* cells (Ganesan, 1974). Experiments to improve the efficiency of endonuclease digestion in *N. crassa* are now under way.

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REFERENCES


Isolation of yeast DNA. Methods in Cellular Biology 12, 39–44.


