Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1354–1358, March 1985
Biochemistry

Regulated expression of endonuclease *Eco*RI in *Saccharomyces cerevisiae*: Nuclear entry and biological consequences

(nuclear localization/*rad52*)

GEORJANA BARNES AND JASPER RINE†

Department of Biochemistry, University of California, Berkeley, CA 94720

Communicated by H. A. Barker, October 24, 1984

ABSTRACT **In an investigation to determine how proteins are localized within the nucleus of a cell, we demonstrate that the restriction endonuclease *Eco*RI is able to enter and function within the nucleus of *Saccharomyces cerevisiae* when this prokaryotic protein is synthesized *in vivo*. The *Eco*RI endonuclease was produced in yeast under the transcriptional control of a regulated yeast promoter by ligating a DNA fragment containing only coding sequences for the endonuclease to the promoter element of the yeast *GAL1* gene (the structural gene for galactokinase, EC 2.7.1.6). Yeast cells harboring a plasmid containing this promoter–gene fusion are able to grow under conditions that repress transcription from the *GAL1* promoter. However, under inducing conditions, these yeast cells are unable to grow. Moreover, *rad52* mutants, which are deficient in the repair of double-strand breaks, are more sensitive to the presence of the promoter–gene fusion plasmid than are wild-type cells. We demonstrate that the *Eco*RI endonuclease activity is present in lysates prepared from yeast transformants grown under conditions that induce transcription of *GAL1*, but this activity is not detectable in cells grown under conditions that repress transcription from the promoter. Furthermore, analysis of yeast chromosomal DNA shows that the endonuclease enters the yeast nucleus and cleaves DNA specifically at *Eco*RI recognition sites.**

Since eukaryotic cells are organized into a variety of membrane-bound organelles, mechanisms should exist to target individual proteins to their final destination in the correct organelle. The best-understood mechanism for protein targeting is the early steps in the synthesis of secreted proteins. These proteins possess an amino-terminal leader that functions as a signal directing the insertion of the proteins into the endoplasmic reticulum (1–3). In addition to this signal, which is part of the nascent polypeptide, there exists an elaborate mechanism in cells for transporting secreted proteins from the endoplasmic reticulum to the outside of the cell (4). In contrast, the mechanism of targeting proteins to the nucleus remains obscure. It is unknown how proteins that reside in the nucleus are moved from the cytoplasm to the nucleus or how these proteins are retained in the nucleus.

One possible route for movement of macromolecules into or out of the nucleus is through the nuclear pores that span the inner and outer nuclear membrane. The pores are complexes of several components whose structure has been resolved for *Xenopus* (5). It remains to be determined whether or not the pore impedes or facilitates the transport of macromolecules between the nucleus and cytoplasm.

Various mechanisms have been proposed for the movement of molecules from the cytoplasm to the nucleus. Injection experiments with artificial macromolecules suggest that diffusion could suffice for entry of molecules with an effective radius of 45–59 Å (6, 7). Both nuclear transplantation experiments (8) and injection experiments with proteins (9) have established some correlation between the size of a protein and its ability to enter and accumulate in the nucleus.

Injection experiments by Dingwall *et al.* (10) using nucleoplasmin, a major nuclear protein of *Xenopus*, have demonstrated that one portion of the protein contains information directing the entry of the entire protein into the nucleus. Furthermore, injection of specific proteolytic fragments of nucleoplasmin demonstrates that one portion of the molecule will remain in the nucleus once injected but will not specifically migrate into the nucleus if injected into the cytoplasm. This result indicates that the accumulation of particular proteins in the nucleus is not simply a result of differential retention of molecules from a pool of molecules passively diffusing into and out of the nucleus.

However, injection experiments in general have intrinsic technical and conceptual limitations (11). In addition to the potential for mechanical damage to the cell and to the material being injected, it is unclear whether a mature protein would be localized upon injection into the cytoplasm by the same mechanisms that operate to target that protein when translated *in situ*.

A genetic approach to the study of nuclear targeting would be feasible if a protein synthesized *in vivo* were lethal to a cell if, and only if, that protein were transported into the nucleus. By using such a protein, mutants could be selected that are deficient in transporting proteins to the nucleus. We have used *Saccharomyces cerevisiae* because of its well-developed genetics to begin a genetic analysis of nuclear targeting. In addition, the requirements for transport of macromolecules into the nucleus may be particularly stringent in yeast because the nuclear membrane does not break down in mitosis (12). As a nucleus-specific lethal protein we have chosen the restriction endonuclease *Eco*RI. The experiments presented here describe the regulated expression of *Eco*RI endonuclease in yeast and demonstrate that this prokaryotic protein is capable of entering the nucleus of yeast and digesting native chromatin at *Eco*RI sites.

MATERIALS AND METHODS

Strains. The strains of *S. cerevisiae* used in these experiments were JRY 438 (*MATα, his4-519, leu2-3, leu2-112, ura3-52*) and JRY 481 (*MATα, rad52-1, arg4, aro7, ura3-52, trp1, ade1, leu2, his4*). The bacterial strain used was MC1061 (13).

Media and Growth Conditions. Yeast-rich medium (YP; yeast extract/peptone) and yeast minimal media (YM) have been described (14). The *rad52-1* mutants were monitored by their inability to grow on YP glucose medium containing 0.05% methyl methanesulfonate. YM medium contained one of three different types of carbon sources: (*i*) 2% glucose for the repressing medium, (*ii*) 3% glycerol/2% potassium ace-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase(s); bp, base pairs.

†To whom reprint requests should be addressed.

tate/40 μg of aspartate per ml in neutral medium, and (*iii*) 2% galactose in inducing medium. Amino acids and bases were added at 30 mg/liter when required. All yeast were grown at 30°C, with those in liquid culture receiving vigorous agitation.

Chemicals. Galactose was purchased from Nutritional Biochemicals. Methyl methanesulfonate was from Eastman Kodak, and [α^{32}P]dCTP was from Amersham. Oligonucleotide linkers (*Bam*HI 10-mers) were from Collaborative Research (Waltham, MA). All other chemicals were from Sigma.

Enzymes. Enzymes were purchased from commercial sources and used according to suppliers' specifications.

Recombinant DNA. Plasmid DNA was prepared as described (15). All *in vitro* DNA manipulations were performed as described (16). For the gel transfer hybridization experiments, DNA was transferred to nitrocellulose filters (Schleicher & Schuell). Autoradiography used Kodak XAR-5 film and Cronex Lightning Plus intensifying screens at −80°C.

Plasmid Constructions. Plasmids containing the *GAL1* promoter and the *Eco*RI structural gene were constructed as diagrammed in Fig. 1. Plasmid pRK26 containing the entire *Eco*RI endonuclease–methylase operon (a gift from Rosalind Kim) was cleaved with *Bam*HI and a 2.7-kilobase (kb) fragment was isolated that included all of the endonuclease gene. This fragment was partially cleaved with *Hin*fI, and a 1.2-kb fragment was isolated that contains the entire endonuclease coding sequence and only 4 base pairs (bp) of the 5′ untranslated sequence; less than half of the methylase gene is present. The 5′ extensions were made flush with a fill-in reaction using DNA polymerase I Klenow fragment. After addition of *Bam*HI linkers (16), the 1.2-kb fragment was cloned into the *Bam*HI site of plasmid pBM125 (a gift from Mark Johnston). pBM125 is a yeast *Escherichia coli* shuttle vector consisting of pBR322, the yeast *URA3* gene, the *ARS1* sequence, the centromere from chromosome IV, and, of particular importance for these experiments, the *GAL1* promoter from yeast (17, 34). The *GAL1* promoter fragment contains the entire control region from the *GAL1* gene and no coding sequence because the 3′ boundary of the fragment lies 4 bp on the 5′ side of the galactokinase (*GAL1* gene) coding region.

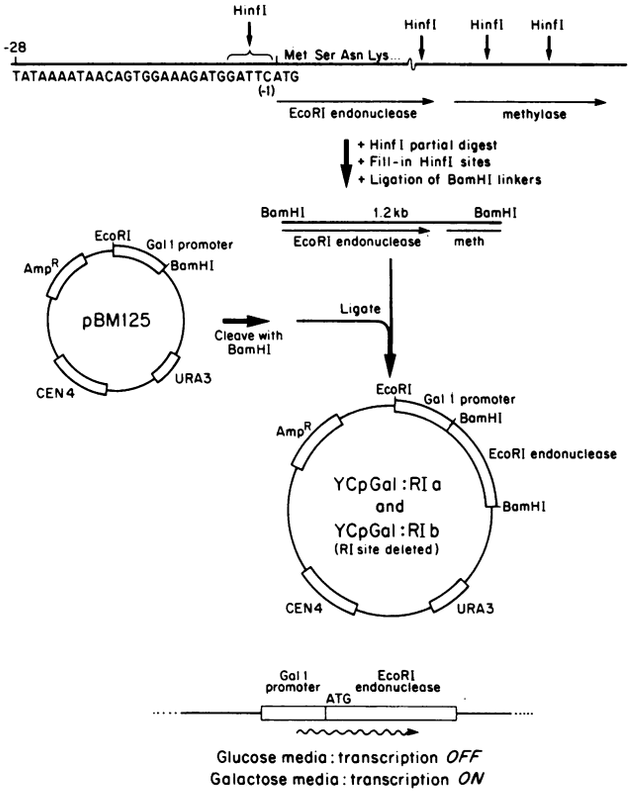

FIG. 1. Construction of the YCpGal:RIa and -b fusion plasmids. The *Eco*RI endonuclease coding sequences were placed under the regulation of the *GAL1* promoter as shown. The mode of transcriptional regulation of the promoter–gene fusion is shown at the bottom. The wavy arrow indicates the direction of transcription of the YCpGal:RI plasmids.

The plasmid generated by this construction, designated YCpGal:RIa, contains a single *Eco*RI recognition site. YCpGal:RIb is identical to YCpGal:RIa except that the unique *Eco*RI recognition site in the YCpGal:RIa plasmid was eliminated by filling in the 5′ overhang after *Eco*RI endonuclease digestion. A control plasmid used in these experiments, referred to as YCpGal:HIS3 consists of the yeast *HIS3* gene under the transcriptional control of the *GAL1* promoter in pBM125 (34).

Transformation. Yeast were transformed by the procedure of Hinnen *et al.* (18) except that spheroplasts were prepared with lyticase (19), a gift from the Schekman lab. *E. coli* were transformed as described in Davis *et al.* (15).

Growth Conditions for Galactose Induction. Single colonies were inoculated into YM medium supplemented with glucose and grown to an A_{600} = 0.7. Cells were harvested, washed once in YM medium with no carbon source, and resuspended in either YM galactose (for shifting from repressing to inducing conditions) or YM glycerol medium (for shifting from nonrepressing to inducing conditions). In the latter experiments, the cells were grown until the cell density doubled in glycerol medium and then were harvested, washed, and resuspended in YM galactose medium as described.

***Eco*RI Endonuclease Assay in Cell Lysates.** Ten milliliters of galactose-induced cells were harvested (A_{600} = 1.5–2.0). All subsequent manipulations were at 0°C. Cells were resuspended in 100 μl of 100 mM NaCl/50 mM Tris, pH 7.4/10 mM $MgSO_4$/1 mg of bovine serum albumin per ml/1 mM phenylmethylsulfonyl fluoride/2 μg of chymostatin per ml/5 mM *p*-aminobenzamidine acid. Glass beads (100 mg, 0.5 mm) were added to each suspension, and the suspensions were mixed in a Vortex until lysis was complete as judged by light microscopy. Cell debris was pelleted, 50- or 100-μl aliquots of the cell supernatants were mixed with 1 μg of λ cI857S7 DNA, and the reaction mixtures were incubated at 37°C. After 1 hr, 5 μl of 0.5 M EDTA was added to each reaction, and the entire reaction was loaded onto a 1% agarose gel and subjected to electrophoresis in the presence of 0.5 μg of ethidium bromide per ml.

Analysis of Nuclear DNA after Galactose Induction. Ten milliliters of galactose-induced cultures was used to isolate DNA. Spheroplasts were lysed by resuspension in 17 mM EDTA, pH 8.5/0.07% $NaDodSO_4$/67% phenol. Cell debris was removed by centrifugation, and DNA was recovered from the supernatant fraction by precipitation with ethanol. To control for possible DNA digestion *in vitro* during the lysis step, 20 units of *Eco*RI endonuclease was added to the EDTA/$NaDodSO_4$ solution before lysis of cells containing the YCpGal:HIS3 plasmid. The isolated DNA was analyzed by gel transfer hybridization experiments.

Analysis of Mitochondrial Function. Yeast cells containing the plasmids were grown in YM glucose medium and induced in YM galactose medium for 10 hr. Aliquots were then plated onto YP glucose plates. After 2 days at 30°C, the resulting single colonies were replica-plated onto YP glycerol plates for evaluation.

RESULTS

A priori one cannot predict whether production of *Eco*RI endonuclease in yeast would affect cell growth. In order to control for this possibility, we constructed a promoter–gene

fusion between the coding sequences for the *Eco*RI endonuclease (20, 21) and the promoter element of the yeast *GAL1* gene (34). This promoter is ideal, as transcription from the promoter is induced by galactose and occurs at a very low level or not at all in media containing noninducing carbon sources such as glycerol. In addition, *GAL1* is also subject to glucose repression: no transcription is detectable from this promoter in cells grown in glucose (22). Furthermore, expression from this promoter is absolutely dependent upon the presence of functional *GAL4* protein, a positive regulator of transcription (23, 24, 17).

The general outline of this construction is shown in Fig. 1, and details are described in *Materials and Methods*. Basically, a restriction fragment was isolated from a clone of the *Eco*RI endonuclease–methylase operon (20) that contained an intact endonuclease coding sequence, a small portion of the methylase gene, and only 4 bp 5′ to the endonuclease initiation codon. The *GAL1* promoter fragment contains the entire control region of the *GAL1* gene except for the 4 bp immediately 5′ to the initiation codon of the *GAL1* gene. A hybrid gene was constructed in a manner that should place *Eco*RI endonuclease under the transcriptional control of *GAL1*. The *GAL1* promoter fragment used in these experiments is on a plasmid designated pBM125. This plasmid consists of pBR322, the *GAL1* promoter fragment, the yeast *URA3* gene for selection, a yeast chromosomal *ARS* element (25), and the centromere of chromosome IV to provide mitotic stability (26). The derivative of pBM125 containing the *Eco*RI endonuclease gene inserted next to the *GAL1* promoter is referred to as YCpGal:RIa and contains a single *Eco*RI recognition site in an area unrelated to the *GAL1* promoter. A second plasmid, YCpGal:RIb, was constructed by mutating the *Eco*RI site in YCpGal:RIa. A third plasmid, YCpGal:HIS3, consists of the *HIS3* gene (27) fused to the *GAL1* promoter on pBM125 and was used as a control plasmid.

YCpGal:RIa, YCpGal:RIb, and the control YCpGal:HIS3 plasmids were transformed into *RAD52* and *rad52* cells. These yeast strains differ in their ability to repair double-strand breaks in DNA. The *rad52* mutation (28) renders yeast 100-fold more sensitive to ionizing radiation than are *RAD52* cells. It appears that in these mutants one double-strand break in DNA is a lethal event (29, 30). Numerous transformants were observed for all three plasmids with either strain when the transformants were selected on glucose-containing medium. When transformants were selected on galactose-containing medium, numerous transformants were obtained with the YCpGal:HIS3 plasmid, but no transformants were obtained with either YCpGal:RIa or YCpGal:RIb plasmids. These results suggest that induction of the *GAL1* promoter in YCpGal:RIa and -b is harmful.

Effects of Carbon Source. The effects of the plasmids on cell growth in media containing carbon sources that either induce or repress transcription of *GAL1* are shown in Fig. 2. In minimal glucose medium, which represses transcription, the plasmids caused no selective growth disadvantage to either the *RAD52* or *rad52* cells. With cells grown on galactose-containing medium, which induces transcription of the *GAL1* promoter, *RAD52* cells containing the YCpGal:RIa plasmid grew approximately as well as cells containing the YCpGal:HIS3 plasmid; yet the same cells containing the YCpGal:RIb plasmid were unable to grow on galactose-containing medium. Since the only difference between the two Gal:R1 plasmids is the presence or absence of an *Eco*RI site, this result indicates that the growth inhibition caused by YCpGal:RI plasmids is likely due to production of active *Eco*RI endonuclease.

In contrast to *RAD52* cells, *rad52* cells were unable to grow in galactose medium with either of the Gal:R1 plasmids, yet grew normally with YCpGal:HIS3 plasmid. Since cells that are deficient in the repair of double-strand breaks in DNA appear to be more sensitive to induction of *Eco*RI expression, we infer that the growth inhibition caused by these plasmids reflects damage caused by *Eco*RI endonuclease to yeast chromosomal DNA. The enhanced potency of YCpGal:RIb relative to YCpGal:RIa is probably due to the unique *Eco*RI site in YCpGal:RIa, rendering this plasmid sensitive to the endonuclease.

The kinetics of cell death are consistent with the time required to induce expression of the *GAL1* promoter (23). Upon galactose induction, cell death of *rad52* mutants containing the YCpGal:RIb plasmid was apparent after 1.5 hr when the cells were pregrown in YM glycerol and after 8 hr when the cells were pregrown in YM glucose.

***Eco*RI Endonuclease Activity in Yeast Lysates.** The results described above predict that upon induction, cells containing a YCpGal:RI fusion plasmid contain active *Eco*RI endonuclease. To test this prediction, lysates prepared from cells containing the YCpGal:RIb plasmid were assayed for endonuclease activity both before and 18 hr after a shift from glucose medium to galactose medium. Endonuclease activity was assayed by mixing phage λ DNA with aliquots of cell lysate (Fig. 3). In lysates from galactose-induced cells, there was a substantial amount of *Eco*RI endonuclease activity, since the pattern of restriction fragments of phage λ DNA was indistinguishable from that obtained with purified enzyme. In additional experiments, *Eco*RI activity was not detectable in cells grown on glycerol medium or in cells containing the YCpGal:HIS3 plasmid grown on galactose medium. Furthermore, *Eco*RI endonuclease activity was detectable at least as early as 6 hr after induction from YM glycerol medium, the time at which the viability of cells containing the YCpGal:RIb plasmid was zero.

***Eco*RI Endonuclease Cleaves Nuclear DNA *in Vivo*.** Although synthesis of *Eco*RI endonuclease activity *in vivo* results in cell death, the above results do not distinguish between death due to cleavage of genomic *Eco*RI sites or to some other unanticipated effect of this protein in yeast. Therefore, we determined whether nuclear DNA is digested *in vivo* in cells containing YCpGal:RI plasmids by a gel transfer hybridization experiment (Fig. 4). Spheroplasts

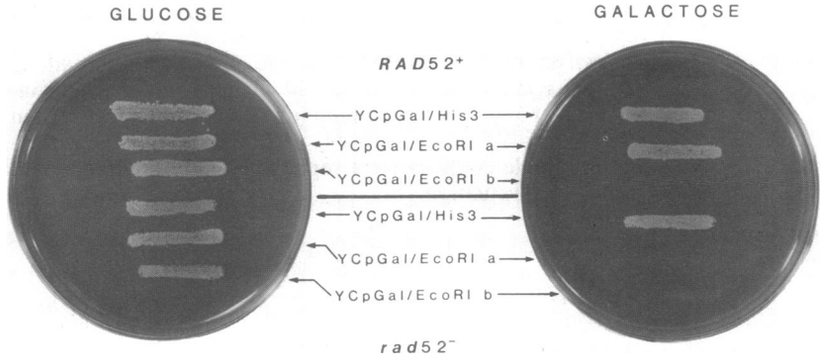

FIG. 2. Growth of yeast transformants. Yeast strains JRY438 (*RAD52*) and JRY481 (*rad52*), transformed with the YCpGal:RIa or -b plasmids or the control YCpGal:HIS3 plasmid, were tested for the ability to grow under conditions inducing or repressing transcription of the *GAL1* promoter. (*Left*) Plate containing YM medium with 2% glucose (repressing conditions). (*Right*) Plate containing YM medium with 2% galactose (inducing conditions). All cells were grown in glucose medium and then duplicate aliquots were plated. The plates were incubated at 30°C for 2 days before being photographed. Note that JRY481 (*rad52*) cells are more sensitive to the presence of the YCpGal:RIa plasmid than are JRY438 (*RAD52*) cells.

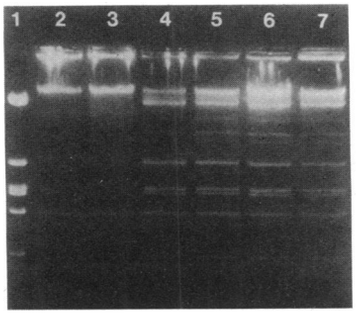

FIG. 3. *Eco*RI endonuclease activity in cell lysates. JRY438 and JRY481 cells transformed with YCpGal:RIb plasmid were grown in medium containing glucose, and then half of each culture was shifted to medium containing galactose and incubated for 18 hr. Lysates were prepared by glass-bead disruption and were assayed for *Eco*RI endonuclease activity by incubating aliquots (50- or 100-μl volumes) with 1 μg of phage λ *c*I857 S7 DNA for 1 hr at 37°C. The reaction was then electrophoresed on a 1% agarose gel. Lanes: 1, control digest of phage λ *c*I857 S7 DNA with legitimate *Eco*RI; 2 and 3, assays of 100-μl aliquots of lysates prepared from JRY438 and JRY481 cells with the YCpGal:RIb plasmid grown in glucose; 4 and 5, 50- and 100-μl aliquots, respectively, of lysates from 438 cells with the YCpGal:RIb plasmid, which were induced in galactose; 6 and 7, 50- and 100-μl aliquots, respectively, of lysates from 481 cells with the YCpGal:RIb plasmid induced in galactose.

were prepared from induced and uninduced cultures, each containing YCpGal:RIa or YCpGal:RIb or YCpGal:HIS3. The spheroplasts were osmotically lysed. DNA was purified from the lysate, subjected to agarose gel electrophoresis, transferred to nitrocellulose, and hybridized to radiolabeled YRp7 DNA. YRp7 contains all of pBR322 sequences and, therefore, is homologous to all three plasmids used in these experiments. In addition, YRp7 was used as a probe because it also contains a fragment of genomic DNA bounded by *Eco*RI sites that contains the yeast *TRP1* gene (25). Therefore, this hybridization probe can be used to measure the

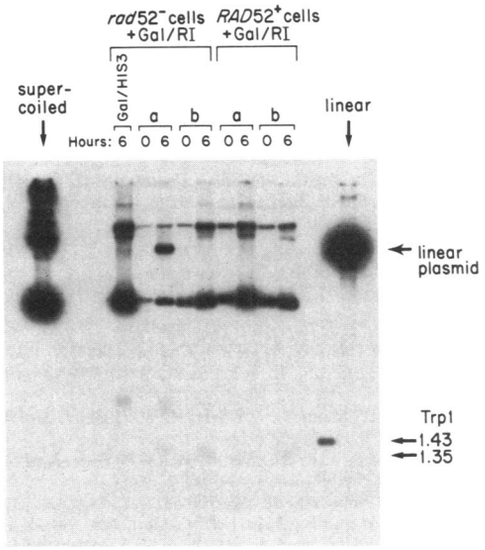

FIG. 4. Analysis of *Eco*RI activity *in vivo*. In a gel transfer hybridization experiment, DNA was isolated from *RAD52* and *rad52* cells containing the YCpGal:RIa or -b plasmid (lanes designated a or b). The cells were induced for 6 hr in galactose medium or were grown under noninducing conditions in glycerol medium (0 hr). The DNA was probed with radiolabeled YRp7 DNA. The top portion of the gel shows that plasmid DNA isolated from all cells at 0 hr is intact and supercoiled. The yeast *TRP1* gene is flanked by *Eco*RI sites in the chromosome, and cleavage of yeast chromosomal DNA *in vitro* with *Eco*RI generates the 1.43-kb *TRP1* gene fragment.

integrity of the plasmids and the fate of the chromosomal *TRP1* gene in cells producing the *Eco*RI endonuclease. To control for the possibility that nuclear DNA was being degraded during the lysis procedure, 20 units of *Eco*RI endonuclease were added to the lysis buffer used to lyse an uninduced culture. We could not detect any digestion of DNA by exogenously added endonuclease.

Plasmid DNA from all uninduced cells (Fig. 4, 0 hr lanes) comigrated with supercoiled plasmid DNA purified from *E. coli* by equilibrium gradient centrifugation. Plasmid DNA from induced *RAD52* cells also comigrated with supercoiled plasmid. However, with DNA from induced *rad52* cells, the YCpGal:RIa plasmid comigrated with YCpGal:RIa DNA that had been linearized *in vitro* with *Eco*RI. In contrast, YCpGal:RIb retained the mobility of a supercoiled plasmid.

The second significant feature of Fig. 4 is apparent near the bottom of the autoradiogram, which shows the *in vivo* effect on chromosomal DNA. Cleavage of purified yeast chromosomal DNA with *Eco*RI *in vitro* generated the *Eco*RI fragment, which the hybridization probe detected as a 1.45-kb *TRP1* fragment (25). In each lane from induced cells that contained a YCpGal:RI plasmid, except *RAD52* cells with the Gal:RIa plasmid, a band was evident that hybridized to the probe near the size of the *TRP1* fragment at 1.35 kb. We believe the slightly smaller size and broader nature of this band relative to the control band (Fig. 4) probably reflects progressive exonuclease digestion of this fragment during the induction time in the experiment. This band was more prominent in DNA from *rad52* cells than from *RAD52* cells and was totally absent in cells containing the YCpGal:HIS3 plasmid. Moreover, when the nitrocellulose filter was reprobed with radiolabeled pBR322, all DNA bands hybridized as before except the 1.45-kb *TRP1* fragment and the 1.35-kb bands, which did not hybridize.

We infer that the *RAD52* cells with the YCpGal:RIa plasmid maintain a supercoiled plasmid after induction and lack the 1.35-kb *TRP1* gene fragment because these cells are able to repair the double-strand breaks caused by the *Eco*RI endonuclease. Furthermore, in repairing the plasmid, these cells probably mutate the plasmid and eliminate the endonuclease activity because lysates made from these cells after a 6-hr galactose induction do not have the *Eco*RI endonuclease activity as measured in the phage λ DNA assay (data not shown). This interpretation is consistent with the ability of these cells to grow in galactose medium as seen in Fig. 2.

From the data presented in the autoradiogram, it is difficult to determine the extent to which the genomic DNA was digested in induced cells containing the YCpGal:RI plasmid. To estimate the extent of cleavage, the gel used in this experiment was stained with ethidium bromide and photographed with ultraviolet illumination prior to transfer of the DNA to nitrocellulose. Due to the small size of the yeast genome, it is possible to recognize a complete digest by inspection for characteristic bands due to multiple molecules of a uniform size from the repeated DNA species such as endogenous yeast plasmid and ribosomal RNA genes. We conservatively estimate that 50% of the *Eco*RI sites are cleaved in induced *rad52* cells.

Effects on Mitochondrial DNA. Functional mitochondrial DNA is required for yeast to grow on a nonfermentable carbon source such as glycerol but is dispensable for growth on fermentable carbon sources such as glucose. In order to determine whether *Eco*RI endonuclease was able to damage mitochondrial DNA at all, cells containing YCpGal:RIb induced for an amount of time sufficient to cause 50% killing were plated out on glucose-containing medium. After the cells were allowed to grow into colonies, the colonies were replica-plated onto glycerol plates to determine whether any had lost the capacity for growth on the nonfermentable carbon source. All 180 colonies tested were capable of growth

on either glucose or glycerol as carbon sources. Therefore, if the endonuclease can enter the mitochondrion and degrade mitochondrial DNA, the enzyme does so only in cells that also have suffered lethal damage to nuclear DNA. It should be noted that the existence of multiple mitochondrial genomes per cell precludes us from determining whether a small number of mitochondrial DNA molecules may be damaged in cells lacking a lethal cleavage in the nuclear DNA.

DISCUSSION

Fusion of the structural gene of *Eco*RI endonuclease to the promoter of the yeast *GAL1* gene on a yeast centromere plasmid has provided us with a mitotically stable plasmid that confers no detectable selective growth disadvantage to cells grown on glucose or neutral carbon sources. Yet upon galactose induction of the promoter, active *Eco*RI endonuclease is made, and the cells that harbor the plasmid die.

Active *Eco*RI endonuclease synthesized *in vivo* is capable of entering the yeast nucleus and killing the cell. Death results from the action of the endonuclease on nuclear genes. This conclusion is derived from the following facts: (*i*) upon induction, active endonuclease is present in cells; (*ii*) the kinetics of killing is consistent with the kinetics of induction of the *GAL1* promoter; (*iii*) *rad52* mutants are much more sensitive to killing by the endonuclease than are *RAD52* cells; and (*iv*) nuclear DNA is digested at *Eco*RI sites and only at *Eco*RI sites. Plasmids lacking *Eco*RI sites remain supercoiled, indicating the absence of *Eco*RI* activity (31).

The entry of the endonuclease into the nucleus has interesting implications for the mechanism(s) by which authentic nuclear proteins enter the nucleus. *Eco*RI endonuclease is a prokaryotic enzyme. Since bacteria lack nuclei, it is unlikely that the endonuclease contains a specific signal to direct its transport to the yeast nucleus. Therefore, it seems plausible that the endonuclease may enter the nucleus through the nuclear pore. The functional radius of the nuclear pore has been measured to be 45–55 Å (6, 7). In principle, this size is sufficient to allow passage of spherical proteins as large as 50–60 kDa. *Eco*RI endonuclease is active as a dimer of 62 kDa and may be able to pass through the pore without the aid of any specific transport apparatus. The results imply that, for some nuclear proteins, there may be no requirement for a specific mechanism to assure entry of a protein into the nucleus. However, for proteins larger than the diameter of the pore or for those proteins that must be concentrated against a gradient, specific mechanisms may exist. It will be interesting to determine if mutations that block entry of the endonuclease into the nucleus (perhaps nuclear pore mutants) also block the entry of authentic nuclear proteins. If fusion proteins can be made between nuclear proteins and *Eco*RI endonuclease that retain endonucleolytic activity, it may be possible to select mutations that inactivate any signal for nuclear targeting that may be present in the protein. This approach may be feasible, as we have recently demonstrated that at least 11 amino acids can be added to the amino terminus of *Eco*RI endonuclease with no loss of *Eco*RI activity (unpublished data).

The *rad52* mutant was isolated by virtue of its sensitivity to ionizing radiation (28). Ionizing radiation, in addition to causing breaks in DNA, also generates free radicals as it passes through aqueous environments (32), thus complicating an interpretation of the properties of the mutant. The differential sensitivity of *rad52* and *RAD52* cells to induction of YCpGal:RIa extends previous work (33, 30) that indicate that the radiation-induced lethality in *rad52* mutants is due to DNA damage *per se* and is not an indirect consequence of secondary effects of ionizing radiation.

The regulated synthesis of the *Eco*RI endonuclease *in vivo* will be useful in a variety of studies, including the mechanism of recombination initiation, negative selections for promoter fusions, and *in vivo* studies of chromatin structure.

We gratefully acknowledge the gifts of pBM125 and YCpGal: HIS3 from Mark Johnston and of pRK26 from Paul Modrich, Rosalind Kim, and Sung Ho Kim. We also thank Paul Modrich, Rosalind Kim, and Sung Ho Kim for useful discussions on the properties of the *Eco*RI endonuclease. We appreciate comments on the manuscript from Michael Basson, Stuart Linn, Jules O'Rear, Donald Rio, and Robert Tjian. In addition, we thank Kerrie Rine for preparation of the figures. This research was supported by National Institutes of Health GM31105 to J.R. and by a National Institute of Environmental Health Sciences Mutagenesis Center grant to the Department of Biochemistry. G.B. was supported by a U.S. Public Health Service Training Grant GM07232.

1. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851.
2. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 852–862.
3. Sabatini, D. D., Kreibich, G., Morimoto, T. & Adesnik, M. (1982) *J. Cell Biol.* **92**, 1–22.
4. Schekman, R. & Novick, P. (1982) in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, eds. Strathern, J. N., Jones, E. W. & Broach, J. B. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 361–393.
5. Unwin, N. P. T. & Milligan, R. A. (1982) *J. Cell Biol.* **93**, 63–75.
6. Reiner, P. (1983) *J. Biol. Chem.* **258**, 11427–11429.
7. Paine, P. L., Moore, L. C. & Horowitz, S. B. (1975) *Nature (London)* **254**, 109–114.
8. Goldstein, L. & Ko, C. (1981) *J. Cell Biol.* **88**, 516–525.
9. DeRobertis, E. M., Longthorne, R. F. & Gurdon, J. B. (1978) *Nature (London)* **272**, 254–256.
10. Dingwall, C., Sharnick, S. V. & Laskey, R. A. (1982) *Cell* **30**, 449–458.
11. Miller, D. S., Lau, Y.-T. & Horowitz, S. B. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1426–1430.
12. Pringle, J. & Hartwell, L. (1981) in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritence*. eds. Strathern, J. N., Jones, E. W. & Broach, J. B. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 97–142.
13. Casadaban, M. J., Martinez-Arias, A., Shapira, S. K. & Chou, J. (1983) *Methods Enzymol.* **100**, 293–308.
14. Barnes, G., Hansen, W., Holcomb, C. & Rine, J. (1984) *Mol. Cell. Biol.* **4**, 2381–2388.
15. Davis, R. W., Botstein, D. & Roth, J. R. (1981) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. St. John, T. P., Scherer, S., McDonell, M. W. & Davis, R. W. (1981) *J. Mol. Biol.* **152**, 317–334.
18. Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929–1933.
19. Scott, J. H. & Schekman, R. (1980) *J. Bacteriol.* **142**, 414–423.
20. Newman, A. K., Rubin, S. H., Kim, S. H. & Modrich, P. (1981) *J. Biol. Chem.* **256**, 2131–2139.
21. Green, P. J., Gupta, M., Boyer, H. W., Brown, W. E. & Rosenberg, J. M. (1981) *J. Biol. Chem.* **256**, 2143–2153.
22. Johnston, S. A. & Hopper, J. E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6971–6975.
23. Adams, B. G. (1972) *J. Bacteriol.* **111**, 308–315.
24. St. John, T. P. & Davis, R. W. (1979) *Cell* **16**, 443–452.
25. Stinchcomb, D. T., Struhl, K. & Davis, R. W. (1979) *Nature (London)* **28**, 39–43.
26. Stinchcomb, D. T., Mann, C. & Davis, R. W. (1982) *J. Mol. Biol.* **158**, 157–179.
27. Struhl, K., Cameron, J. R. & Davis, R. W. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1471–1475.
28. Resnick, M. A. (1969) *Genetics* **62**, 519–531.
29. Ho, K. S. Y. & Mortimer, R. K. (1973) *Mutat. Res.* **20**, 45–51.
30. Strathern, J. N., Klar, A. J. S., Hicks, J. B., Abraham, J. A., Ivy, J. M., Nasmyth, K. A. & McGill, C. (1982) *Cell* **31**, 183–192.
31. Polinsky, B., Green, B., Garfin, D. E., McCarthy, B. J., Goodman, H. M. & Boyer, H. W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3310–3314.
32. Benon, H., Beilski, J. & Gebicki, J. M. (1977) in *Free Radicals in Biology*, ed. Pryor, W. A. (Academic, New York), Vol. 111.
33. Malone, R. E. & Esposito, R. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 503–507.
34. Johnston, M. & Davis, R. W. (1984) *Mol. Cell. Biol.* **4**, 1440–1448.