Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6249–6253, July 1995
Genetics

Interaction with the recombination hot spot χ *in vivo* converts the RecBCD enzyme of *Escherichia coli* into a χ-independent recombinase by inactivation of the RecD subunit

(exonuclease V/DNA degradation/RecA)

ANDREAS KÖPPEN, SYLVIA KROBITSCH*, BRIGITTE THOMS, AND WILFRIED WACKERNAGEL†

Genetik, Fachbereich Biologie, Universität Oldenburg, Postfach 2503, D-26111 Oldenburg, Germany

Communicated by Franklin W. Stahl, University of Oregon, Eugene, OR, February 16, 1995

ABSTRACT **The RecBCD enzyme of *Escherichia coli* promotes recombination preferentially at χ nucleotide sequences and has *in vivo* helicase and strong duplex DNA exonuclease (exoV) activities. The enzyme without the RecD subunit, as in a *recD* null mutant, promotes recombination efficiently but independently of χ and has no nucleolytic activity. Employing phage λ *red gam* crosses, phage T4 2^- survival measurements, and exoV assays, it is shown that *E. coli* cells in which RecBCD has extensive opportunity to interact with linear χ-containing DNA (produced by rolling circle replication of a plasmid with χ or by bleomycin-induced fragmentation of the cellular chromosome) acquire the phenotype of a *recD* mutant and maintain this for ≈2 h. It is concluded that RecBCD is converted into RecBC during interaction with χ by irreversible inactivation of RecD. After conversion, the enzyme is released and initiates recombination on other DNA molecules in a χ-independent fashion. Overexpression of *recD*$^+$ (from a plasmid) prevented the phenotypic change and providing RecD after the change restored χ-stimulated recombination. The observed *recA*$^+$ dependence of the downregulation of exoV could explain the previously noted "reckless" DNA degradation of *recA* mutants. It is proposed that χ sites are regulatory elements for the RecBCD to RecBC switch and thereby function as cis- and trans-acting stimulators of RecBC-dependent recombination.**

The RecBCD enzyme is an essential component of the main homologous recombination pathway in *Escherichia coli* (see refs. 1 and 2). This pathway recombines linear DNA molecules during conjugation, transduction, and vegetative phage crosses and is required for repair of double-strand breaks (3, 4). RecBCD enzyme is an ATP-dependent exonuclease (exoV) that consists of three protein subunits encoded by the *recB*, *recC*, and *recD* genes. ExoV activity is composed of ATP-dependent DNA helicase and single-stranded DNA endonuclease activities of RecBCD enzyme acting on linear double-stranded DNA to produce single-stranded DNA oligonucleotides (see ref. 5). In cells and in cell-free extracts with ATP, RecBCD provides the major linear duplex DNA-degrading activity (6–8).

χ hot spots of RecBCD-dependent recombination are present in the *E. coli* genome ≈1000 times and stimulate, when present in phage λ, recombination in λ *red gam* crosses with decreasing efficiency in increasing distance on the left side of their sequence 5′-GCTGGTGG-3′ as written here (see ref. 9). Hot spot activity is exerted only when RecBCD enters the DNA molecule from a double-stranded end placed on the 3′ side of χ (see ref. 9). An important finding was that *recD* null mutants were recombination proficient and as UV-resistant as wild-type cells (10, 11). They had lost all nucleolytic activities of RecBCD, and recombination was χ independent (10, 11). Moreover, λ *red gam* crosses established that recombination in *recD* mutants was fully dependent on *recC*$^+$ and that exchange events were focused at double-stranded ends of DNA (12). Thaler *et al.* (12, 13) proposed that by interaction with χ RecBCD changes from a nuclease to a recombinase and that this switch results from the dissociation of RecD. Subsequently, it was found that RecBC(D$^-$) enzyme has DNA helicase activity *in vitro* (14–16) and *in vivo* (17). The assumed loss of RecD at χ was incorporated into models (9, 18) in which RecBCD loads on a duplex DNA end, degrades both strands until it meets a properly oriented χ site, and then is changed into a helicase that further unwinds the molecule (17–20). The 3′ and 5′ single strands are both available for RecA-promoted strand invasion (18, 21–23).

χ sequences in linear plasmid DNA protect it against RecBCD *in vivo* (20, 24). The attenuation *in vitro* at χ of the nuclease but not helicase activity of RecBCD suggested a regulation of RecBCD by χ (19, 25). The nuclease attenuation is reversed by a change of the reaction conditions (16). Evidence for a χ-triggered dissociation of RecD has not yet been found (16).

What is the status of RecBCD in cells upon interacting with χ-containing DNA? The following possibilities can be tested experimentally: (*i*) The enzyme is trapped on the DNA molecule, perhaps at χ; (*ii*) the enzyme loses its RecD subunit upon encountering χ and regains it upon exit from the DNA; (*iii*) the enzyme changes at χ as in *ii* but does not reassemble after release from DNA. To monitor the status of RecBCD *in vivo* we used λ *red gam* crosses to quantify χ-specific recombination (χ activity) and overall recombination (11). This allowed us to distinguish the wild type (high χ activity and recombination proficiency) from *recB* (no χ activity and low recombination proficiency) and from *recD* (no χ activity and high recombination proficiency) mutant phenotypes.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *E. coli* AB1157 (referred to as wild type) and its *recB21* (WA632), *recD1011* (BT125), and *lexA3* (WA426) derivatives have been described (17, 26). A Δ(*srl-recA*)*306*::Tn*10* mutation was also transduced into AB1157 (WA826) (27). The plasmids pINDχ^0, pINDχ^1, pINDχ^2 (spectinomycin resistance), and pHelper (ampicillin resistance) were those of Dabert *et al.* (24). Cells of AB1157 were transformed with combinations of pIND plus pHelper (24). Plasmid pSK1 contains the 3.8-kb *Pst* I fragment covering the *recD*$^+$ gene from pPB120 (26) downstream of the *tac* promoter in the expression vector pJF118EH (28). When BT125 with pSK1 was grown in LB broth with isopropyl

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: Bm, bleomycin; exoV, exonuclease V; e.o.p., efficiency of plating; IPTG, isopropyl β-D-thiogalactopyranoside.

*Present address: Bernhard-Nocht-Institut für Tropenmedizin, Bernhard-Nocht-Strasse 74, D-20359 Hamburg, Germany.
†To whom reprint requests should be addressed.

β-D-thiogalactopyranoside (IPTG; 1–10 mM), a protein band of ≈67 kDa was seen when total cell proteins were separated on SDS/PAGE. The protein was not seen in extracts from AB1157 or BT125 or from AB1157 with pSK1 grown in the presence of <0.1 mM IPTG.

Media and Plating Conditions. For λ crosses logarithmic-phase cells were grown in LB broth with 0.2% maltose. Incubation temperatures for cultures and platings on LB-agar were 30°C unless stated otherwise. The plating of T4 2^- (7) was done as described (17) except that after preadsorption of phage 0.01 ml of an overnight culture of AB1157 was added as indicator.

Treatment of Cells with Bleomycin (Bm). A sample from a concentrated stock solution of Bm sulfate (courtesy of H. Mack Nachfolger, Karlsruhe, Germany) was added to a logarithmic-phase LB broth culture (20 μg/ml). Aeration was continued at 30°C for the indicated times. Then the cells were sedimented by 30 sec of centrifugation at 15,000 × *g* and resuspended in prewarmed LB broth.

Determination of χ Activity. The χ activity was determined in λ *red gam* crosses as described (26) by the method of Stahl and Stahl (29). In the crosses χ activity is measured by the higher recombination frequency in a genomic region containing χ compared to a genomic region not containing χ. The burst sizes were between 10 and 70 and in Bm-treated cells they were between 3 and 10. In cells overexpressing *recD*$^+$ and treated with Bm, the burst size was ≈1 when the cross was performed immediately after treatment.

Determination of exoV Activity. Cell-free extracts (30) were cleared by centrifugation at 28,000 × *g* for 15 min at 4°C. The exoV activity was determined (31) by using [^{3}H]thymidine-labeled P22 DNA (specific radioactivity, 4.5 × 10^6 cpm/μmol) and the following reaction conditions: 50 mM Tris·HCl, pH 7.5/10 mM $MgSO_4$/1 mM dithiothreitol/10% (vol/vol) glycerol/0.5 mg of bovine serum albumin per ml/60 mM KCl/0.3 mM ATP. Reactions were carried out at 37°C for 30 min. One unit of exoV activity releases 1 nmol of acid-soluble DNA fragments in 30 min under the conditions described.

RESULTS

Partial *recD* Mutant Phenocopy Caused by Rolling Circle Replication of Plasmids with χ. χ sequences in linear plasmid replication intermediates protect the DNA against degradation by RecBCD (24). In these experiments, the target plasmid (pIND) contained a temperature-sensitive θ replicon and a rolling circle origin of replication dependent on Rep protein. The helper plasmid (pHelper) contained the *rep* gene under the control of the thermoinducible λ P_L promoter. A shift from 28°C to 40°C induced rolling circle replication of the target plasmid. In *recBCD*$^+$ cells, linear replication intermediates accumulated only when the target plasmid contained a χ site and mainly when the 3′ side of χ was oriented toward the DNA end upon which RecBCD enzyme can load (24). The protection of linear DNA could result from trapping of RecBCD (*recB* mutant phenocopy) or from conversion of RecBCD into an entity devoid of exoV activity (refs. 12 and 13; *recD* mutant phenocopy).

λ *red gam* crosses were performed in cells in which the rolling circle replication system of Dabert *et al.* (24) was switched on. Under conditions where plasmid replication intermediates were not protected because of the lack of χ sites (Table 1, experiment 1) or were hardly protected because of wrongly oriented χ sites (Table 1, experiment 2), the χ activity was high. In contrast, a significantly lower χ activity was seen in cells where properly oriented χ sites protected replication intermediates (Table 1, experiment 3). In the three experiments, the frequency of λ J^+R^+ recombinants was the same (Table 1). The unimpaired recombination and decreased χ activity in experiment 3 argue against the trapping explanation and are compatible with RecBCD conversion leading to a *recD* mutant phenocopy. This interpretation implies that after χ-triggered conversion the enzyme is released from the DNA to interact with other DNA molecules (see below). The still considerable level of χ activity in experiment 3 could reflect an insufficient amount of linear replication intermediates for conversion of all RecBCD. This is in accord with the finding that in wild-type cells the protection by χ never yielded the high amounts of replication intermediates found in *recB* cells (24).

Table 1. Effect of χ sites in linear plasmid replication intermediates on χ activity and recombinant formation in λ *red gam* crosses

Strain	Plasmid	χ activity	% J^+R^+ recombinants
AB1157	pINDχ^0 pHelper	6.36 ± 0.36	5.59 ± 1.94
AB1157	pINDχ^1 pHelper	5.91 ± 0.13	5.47 ± 1.36
AB1157	pINDχ^2 pHelper	4.63 ± 0.26	5.32 ± 1.55

Logarithmic-phase cells were grown and λ *red gam* crosses were performed at 40°C to induce and maintain rolling circle replication of the pIND plasmids (24). χ^0, no χ site present; χ^1, 3′ side of χ directed away from the end of replicated linear DNA; χ^2, 3′ side of χ directed toward the end of replicated linear DNA. Data from three independent experiments are given with SD (comparison of the χ activity in the χ^0 and χ^2 experiments by *t* test gave $t = 9.5$ and $P = 0.001$).

Bm Treatment Produces DNA Substrates for RecBCD *in Vivo*. To increase the number of χ-containing DNA fragments with which RecBCD can interact, we fragmented the *E. coli* chromosome itself. Cells were treated with Bm, which induces double-strand breaks in DNA, most of which are flush ended or nearly flush ended (32) and are suitable entry sites for RecBCD (31, 33). An indication that RecBCD loads on the Bm-induced DNA ends would be an increase of the survival of phage T4 2^-. Due to a mutation in gene *2*, this phage lacks the pilot proteins on its genomic ends, making the phage sensitive to RecBCD (7). Compared to its plating on a *recB* null mutant the efficiency of plating (e.o.p.) of T4 2^- on AB1157 cells is 4 × 10^{-4} (17). This e.o.p. is increased ≈700-fold after 10 min of Bm treatment (Fig. 1*a*). At this dose, the cellular survival was ≈22% and the e.o.p. of T4$^+$ was unaffected. Pulsed-field gel electrophoresis of DNA from the Bm-treated cells showed a

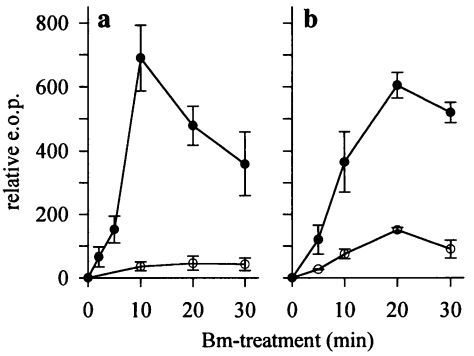

FIG. 1. The e.o.p. of T4 2^- on *E. coli* strains treated with Bm for various time periods relative to untreated cells (relative e.o.p.). (*a*) AB1157 (●) and its Δ*recA* derivative WA826 (○). (*b*) AB1157 pJF118EH (●) and AB1157 pSK1 (*recD*$^+$) (○). Logarithmic-phase cells of the plasmid-containing strains were grown in LB broth with IPTG (1 mM) and the soft agar contained IPTG (5 mM). Relative e.o.p. is the plaque titer on Bm-treated cells divided by the plaque titer on nontreated cells. The e.o.p. of T4 2^- on cells not treated with Bm were 4 × 10^{-4} (AB1157), 5 × 10^{-4} (AB1157 with pJF118EH or pSK1), and 2 × 10^{-3} (WA826) relative to WA632 (*recB*). Data in *a* are given with SD ($n = 3$) and in *b* are given with deviations from the mean ($n = 2$).

smear of DNA fragments between 50 and 500 kbp, indicating *in vivo* fragmentation of chromosomal DNA (data not shown). These results corroborate an earlier finding on increased survival of T4 2^- on cells in which DNA double-strand breaks were induced by γ-irradiation (34).

χ Activity but Not Recombination Is Abolished in Bm-Treated Cells. λ *red gam* crosses were performed in Bm-treated cells (Table 2). In AB1157 the χ activity was abolished, as it is in *recB* or *recD* mutants. However, the J^+R^+ recombinant frequency was only somewhat lower than that in a *recD* and much higher than that in a *recB* mutant. Bm treatment of *recB* or *recD* mutants did not alter their χ activity and only slightly decreased recombination in the *recD* strain, suggesting that the phenotypic change seen in the wild-type cells relied on RecBCD (Table 2). The elimination of χ activity and maintenance of recombination suggest that chromosomal fragmentation led not to trapping but to conversion of RecBCD. This parallels the conclusions drawn from the data in Table 1 and explains the survival increase of T4 2^- (Fig. 1*a*). It is possible that a fraction of the RecBCD and presumptive RecBC complexes interacted with chromosomal DNA fragments, which caused the somewhat lower recombination frequencies in Bm-treated wild-type and *recD* cells.

Overproduction of RecD Protein Prevents Bm-Induced Loss of χ Activity. If the phenotype of Bm-treated cells results from the inactivation or removal of RecD from RecBCD, then overproduction of RecD might counteract the formation of the *recD* mutant phenocopy. This was observed (Fig. 2). In IPTG-treated AB1157 cells containing plasmid pSK1 with the IPTG-inducible $recD^+$ gene, χ activity did not decline upon Bm treatment for 1 or 2 min, which completely eliminated χ activity in AB1157 with the vector plasmid (Fig. 2). These findings can be interpreted to mean that RecBCD in the presence of excess RecD either does not lose its RecD subunit during interaction with χ or that the enzyme can be refurnished with new RecD. Observations in support of the second possibility are reported below.

Overproduction of RecD Protein and a Δ*recA* Mutation Reduce the Bm-Induced Increase of T4 2^- Survival. In cells overproducing RecD the Bm treatment caused a lower increase of the e.o.p. of T4 2^- than in wild-type cells (Fig. 1*b*), which could reflect a higher level of RecBCD. This would support the interpretation that RecBC can be refurnished with RecD and thereby regain exoV activity. The observation that the Bm-induced increase of the e.o.p. of T4 2^- is strongly reduced in a Δ*recA* mutant (Fig. 1*a*) argues for an important role of RecA in the attenuation of exoV.

ExoV Activity in Extracts of Bm-Treated Cells. The increased e.o.p. of T4 2^- and the elimination of χ activity in Bm-treated cells both suggested a low level of exoV activity. In fact, only 3% of the exoV activity present in extracts of AB1157 cells was found when the cells were treated with Bm before extract preparation (Table 3). In cells overproducing RecD, a less drastic activity decrease was evident (Table 3). These observations suggest that the Bm-induced decrease of exoV activity is related to insufficient availability of RecD, but they do not allow us to distinguish whether excess of RecD prevents loss of RecD or refurnishes RecBC with new RecD to regain exoV activity. The possibility that during extract preparation much of RecBCD was removed together with cellular DNA in the high-speed centrifugation was excluded by the following observation. A polymin P fractionation and ammonium sulfate elution (35) of the centrifugation sediments recovered only 0.2–0.8% of the exoV activity present in the extracts from the extract sediments of Bm-treated cells and <0.1% from the extract sediments of untreated cells.

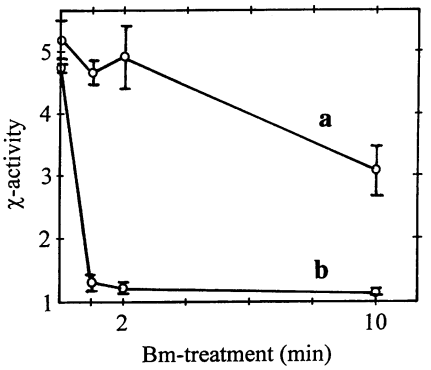

FIG. 2. χ activity in cells treated with Bm for various time periods before λ *red gam* crosses. Curve a, AB1157 with pSK1 ($recD^+$); curve b, AB1157 with vector pJF118EH. Numbers below give percentage J^+R^+ recombinants for each time point. Cells were grown in LB broth with IPTG (1 mM). Data are means from two independent experiments; bars indicate deviations from the mean.

Restoration of χ Activity by Derepression of $recD^+$. In the following experiments, we examined whether the derepression of $recD^+$ on pSK1 would restore χ activity that had been eliminated by Bm treatment. In AB1157 with vector (control), almost no recovery of χ activity was seen within 2 h after Bm treatment and some recovery was seen in cells with pSK1 only after 2 h, whether IPTG induced or not (Fig. 3). This was not surprising, considering the previous finding that treatment of cells with DNA-damaging agents wipes out χ activity 40–120 min later as a result of SOS gene derepression (26, 36), particularly of *ruvA* and *ruvB* (M. Braunschweiger and W.W., unpublished data) and perhaps other genes. Thus, even if RecBCD were regenerated after IPTG-induced RecD overproduction, it would not be visible as a prompt increase of χ activity. In *lexA3* cells in which induction of the SOS system is prevented, χ activity did not decline after UV or mitomycin C treatment (26), but it decreased immediately upon DNA fragmentation by Bm, most probably because of RecBCD conversion (Fig. 3). In Bm-treated *lexA3* pSK1 cells, the derepression of $recD^+$ led to a rapid recovery of χ activity (Fig. 3). The presumptive RecBC enzyme in Bm-treated cells ap-

Table 2. χ activity and J^+R^+ recombinant formation in λ *red gam* crosses performed in wild-type and *rec* mutants of *E. coli* without and with Bm treatment

Strain	*rec* genotype	Bm treatment	χ activity	% J^+R^+ recombinants
AB1157	Wild type	−	5.1 ± 0.7	8.7 ± 2.4
WA632	*recB21*	−	1.3 ± 0.2	0.3 ± 0.1
BT125	*recD1011*	−	1.0 ± 0.2	6.4 ± 1.8
AB1157	Wild type	+	1.4 ± 0.4	3.6 ± 0.7
WA632	*recB21*	+	1.2 ± 0.3	0.4 ± 0.1
BT125	*recD1011*	+	1.1 ± 0.1	4.0 ± 0.4

Cells were treated with Bm for 2 min. Data are given with SD (n = 3–8).

Table 3. Activity of exoV in cell extracts

Strain	Bm treatment	ExoV activity, units/mg
AB1157 pJF118EH	−	64.9 ± 7.6
AB1157 pJF118EH	+	2.2 ± 1.2
AB1157 pSK1 ($recD^+$)	−	62.4 ± 9.1
AB1157 pSK1 ($recD^+$)	+	15.6 ± 7.8
BT125 (*recD1011*) pSK1 ($recD^+$)	−	51.0
BT125 (*recD1011*) pSK1 ($recD^+$)	+	19.4

Cells were grown in the presence of IPTG (1 mM) treated with Bm for 2 min before extract preparation. Data are given with SD from three independent experiments.

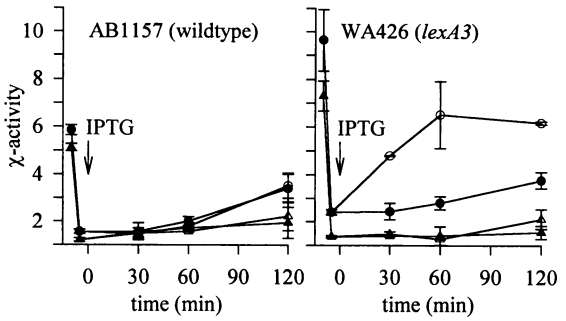

FIG. 3. χ activity in AB1157 (wild type) and WA426 (*lexA3*) cells treated with Bm for 5 min and then incubated for various time periods in LB broth with IPTG added (1 mM; arrow; open symbols) or without IPTG (solid symbols). Cells contained vector pJF118EH (triangles) or pSK1 (*recD*$^+$) (circles). Data are means from two independent experiments; bars indicate deviations from the mean.

pears ready to associate with RecD when available. The partial recovery of χ activity in AB1157 pSK1 seen after 2 h can be explained by assuming that after 2 h the SOS-induced block of χ-specific recombination is relieved and that the background expression of *recD*$^+$ from the multicopy plasmid suffices to keep up χ-specific recombination by regenerating any newly converted enzyme to RecBCD.

DISCUSSION

We show that wild-type cells of *E. coli* in which RecBCD has extensive opportunity to interact with χ-containing DNA (chromosomal DNA fragments or linear plasmid multimers) change their phenotype toward that of a *recD* mutant. The new phenotype is manifest in *recB*-dependent, χ-independent recombination proficiency (measured in λ *red gam* crosses) and low exoV activity (measured by the e.o.p. of T4 *2*$^-$ and exoV activity in cell extracts). This phenotype is not compatible with a permanent trapping of RecBCD on duplex DNA. We conclude that the conversion of RecBCD upon interaction with χ affects RecD, which is required for χ-specific recombination and exoV activity (10, 11). Additional support for the conversion model comes from the findings that the presence of excess RecD prevents the phenotypic change (Fig. 2; Table 3) and that supply of fresh RecD after the phenotypic change reverts the change by restoring χ-specific recombination proficiency (Fig. 3). The proposed conversion model contains essential parts of the previous postulate of Thaler *et al.* (13) that an "encounter between RecBCD enzyme and a χ sequence dissociates the RecD subunit from the holoenzyme" (13).

What happens to RecD during conversion of RecBCD? Since the *recD* mutant phenocopy is maintained for up to 2 h and reverts to wild-type phenotype only when extra RecD is provided (Figs. 2 and 3), we conclude that conversion is not a reversible dissociation of RecD but an irreversible inactivation of RecD (or permanent sequestration). The acquired χ-independent recombination proficiency of the cells suggests that the resulting RecBC detaches from DNA during or shortly after conversion and does not reunite with its RecD (presumably because that is inactivated), although the RecD binding site(s) is free for association with a new RecD (Fig. 3). The reversible change of RecBCD at χ seen *in vitro* (16, 19, 25) is possibly part of a process during which *in vivo* the irreversible conversion of the enzyme is achieved. This could involve cellular components not present in the *in vitro* experiments.

The data in Fig. 1*a* strongly suggest that conversion depends on *recA*$^+$. The role of RecA could be indirect by acting in the derepression of an SOS gene necessary for conversion. In a *lexA3* mutant, in which the induction of SOS genes is blocked, a higher increase of T4 *2*$^-$ survival after Bm treatment was seen than in the Δ*recA* mutant (A.K., B.T., and W.W., unpublished data), suggesting that RecA and not the SOS induction is required for conversion of RecBCD. A *recA*-dependent inducible gene not controlled by LexA protein could also be considered to cause the conversion (37). The protection of χ-containing DNA against RecBCD also depends on RecA (20, 24). If the protection results from attenuation of exoV and attenuation is caused by RecD inactivation as suggested here, then RecA could be directly involved in producing the attenuation, perhaps by interaction with RecBCD. Evidence for a physical cooperation during recombination between RecA and RecBCD *in vivo* has recently been provided (38). Unwound DNA behind RecBCD could activate the coprotease of RecA (36) and thereby focus it to RecBCD, pausing at χ. In accord with this assumption, the increase of T4 *2*$^-$ survival in Bm-treated cells of the recombination-deficient *recA142* mutant was higher than in Bm-treated cells of the Δ*recA* mutant (Fig. 1*a*; unpublished data). The RecA142 protein has residual coprotease activity (5).

The inference that RecA downregulates exoV via χ-dependent inactivation of RecD could explain several previous findings on the effect of *recA* mutations on DNA damage-induced DNA degradation in *E. coli*. (*i*) The postulated SOS-inducible inhibitor of exoV (39) could be RecA itself, which is synthesized in higher amounts in damaged cells and therefore would silence RecBCD during chromosomal DNA degradation more effectively. This would also explain why previous *in vitro* searches for protection of DNA against RecBCD by binding of RecA or other inducible proteins to DNA before addition of exoV were either unsuccessful or did not give conclusive results (31, 40, 41). These studies did not simulate the conditions that lead to downregulation of exoV as proposed here. (*ii*) The *recB*$^+$*C*$^+$*D*$^+$-dependent "reckless" degradation of chromosomal DNA in *recA* mutants after DNA damage (3) could be due to the absence of RecA as the exoV silencer. The similar reckless phenotype of cells overproducing RecD (42) is easily explained because excess RecD counteracts the downregulation of exoV; these cells are sensitive to DNA double-strand breaking agents including γ-irradiation (42) and Bm (unpublished results). (*iii*) Degradation of DNA lacking χ sites should be reckless in wild-type and *recA* cells. It was found that γ-irradiated T4 DNA is degraded in wild-type as effectively as in *recA* cells (43). T4 has <1/10th the χ-site density of *E. coli* (3 per 166 kb; E. Kutter, personal communication). It is not known whether these sites are recognized in DNA with glycosylated hydroxymethylcytosine.

In our experiments, the χ-containing DNA molecules triggering conversion of RecBCD to RecBC were other than those with which the χ-independent recombination proficiency of the cells was measured. We conclude that χ sites act not only in cis by stimulating exchanges close to χ but also in trans. The trans effect relies (*i*) on the release of converted enzyme from the χ-containing DNA and (*ii*) on the χ-independent recombinagenic action on other DNA substrates. The converted enzyme, which has the exoV$^-$ properties of RecBC, is probably the cause of "RecBCD titration" (24) and of the protection of linear plasmid DNA not containing χ afforded by linearized plasmid containing χ (20). The proposed conversion also suggests an efficient recombinational repair in *E. coli* of DNA double-strand breaks according to the model of Szostak *et al.* (44): RecBCD could load on one duplex DNA end at a break, would move to the first χ site (possibly with DNA degradation), would be converted while initiating strand exchange, would be released, and then could interact with another duplex DNA end where it can initiate recombination without degradation and the need for a χ site. This scheme seems particularly advantageous if cells have received several DNA double-strand breaks.

We thank P. Dabert and A. Gruss for providing plasmids. The help of S. Steppat and M. Braunschweiger with some of the experiments is gratefully acknowledged. This work was supported by the Fonds der Chemischen Industrie.

1. Clark, A. J. (1973) *Annu. Rev. Genet.* **7,** 67–86.
2. Smith, G. R. (1988) *Microbiol. Rev.* **52,** 1–28.
3. Willetts, N. S. & Clark, A. J. (1969) *J. Bacteriol.* **100,** 231–239.
4. Sargentini, N. J. & Smith, K. C. (1986) *Radiat. Res.* **107,** 58–72.
5. Kowalczykowski, S., Dixon, D. A., Eggleston, A. K., Lauder, S. D. & Rehrauer, W. M. (1994) *Microbiol. Rev.* **58,** 401–465.
6. Simmon, V. F. & Lederberg, S. (1972) *J. Bacteriol.* **112,** 161–169.
7. Oliver, D. B. & Goldberg, E. B. (1977) *J. Mol. Biol.* **116,** 877–881.
8. Barbour, S. D. & Clark, A. J. (1970) *Proc. Natl. Acad. Sci. USA* **65,** 955–961.
9. Myers, R. S. & Stahl, F. W. (1994) *Annu. Rev. Genet.* **28,** 49–70.
10. Chaudhury, A. M. & Smith, G. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7850–7854.
11. Amundsen, S. K., Taylor, A. F., Chaudhury, A. M. & Smith, G. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5558–5562.
12. Thaler, D. S., Sampson, E., Siddiqi, I., Rosenberg, S. M., Thomason, L. C., Stahl, F. W. & Stahl, M. M. (1989) *Genome* **31,** 53–67.
13. Thaler, D. S., Sampson, E., Siddiqi, I., Rosenberg, S. M., Stahl, F. W. & Stahl, M. M. (1988) in *Mechanisms and Consequences of DNA Damage Processing*, eds. Friedberg, E. & Hanawalt, P. (Liss, New York), pp. 413–422.
14. Masterson, C., Boehmer, P. E., McDonald, F., Chaudhuri, S., Hickson, I. D. & Emmerson, P. T. (1992) *J. Biol. Chem.* **267,** 13564–13572.
15. Korangy, F. & Julin, D. A. (1993) *Biochemistry* **32,** 4873–4880.
16. Dixon, D. A., Churchill, J. J. & Kowalczykowski, S. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2980–2984.
17. Rinken, R., Thoms, B. & Wackernagel, W. (1992) *J. Bacteriol.* **174,** 5424–5429.
18. Rosenberg, S. M. & Hastings, P. J. (1991) *Biochimie* **73,** 385–397.
19. Dixon, D. A. & Kowalczykowski, S. C. (1991) *Cell* **66,** 361–371.
20. Kuzminov, A., Schabtach, E. & Stahl, F. W. (1994) *EMBO J.* **13,** 2764–2776.
21. Dutreix, M., Rao, B. J. & Radding, C. M. (1991) *J. Mol. Biol.* **219,** 645–654.
22. Rosenberg, S. M. (1988) *Genetics* **119,** 7–21.
23. Konforti, B. B. & Davis, R. W. (1992) *J. Mol. Biol.* **227,** 38–53.
24. Dabert, P., Ehrlich, S. D. & Gruss, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12073–12077.
25. Dixon, D. A. & Kowalczykowski, S. C. (1993) *Cell* **73,** 87–96.
26. Rinken, R. & Wackernagel, W. (1992) *J. Bacteriol.* **174,** 1172–1178.
27. Ihara, M., Oda, Y. & Yamamoto, K. (1985) *FEMS Microbiol. Lett.* **30,** 33–35.
28. Fürste, J. P., Pansegrau, W., Frank, R., Blöcker, H., Scholz, P., Bagdasarian, M. & Lanka, E. (1986) *Gene* **48,** 119–131.
29. Stahl, F. W. & Stahl, M. M. (1977) *Genetics* **86,** 715–725.
30. Unger, R. C. & Clark, A. J. (1972) *J. Mol. Biol.* **70,** 539–548.
31. Prell, A. & Wackernagel, W. (1980) *Eur. J. Biochem.* **105,** 109–116.
32. Povirk, L. F. & Austin, M. J. F. (1991) *Mutat. Res.* **257,** 127–143.
33. Taylor, A. F. & Smith, G. R. (1985) *J. Mol. Biol.* **185,** 431–443.
34. Brcic-Kostic, K., Salaj-Smic, E., Marsic, N., Kajic, S., Stojiljkovic, I. & Trgovcevic, Z. (1991) *Mol. Gen. Genet.* **228,** 136–142.
35. Jendrisak, J. J. & Burgess, R. R. (1975) *Biochemistry* **14,** 4639–4644.
36. Little, J. W. & Mount, D. W. (1982) *Cell* **29,** 11–22.
37. Kannan, P. & Dharmalingam, K. (1990) *Curr. Microbiol.* **21,** 7–15.
38. de Vries, J. & Wackernagel, W. (1992) *J. Gen. Microbiol.* **138,** 31–38.
39. Pollard, E. C. & Randall, E. P. (1973) *Radiat. Res.* **55,** 265–279.
40. Prell, A. & Wackernagel, W. (1981) *J. Biol. Chem.* **256,** 10415–10419.
41. Williams, J. G. K., Shibata, T. & Radding, C. M. (1981) *J. Biol. Chem.* **256,** 7573–7582.
42. Brcic-Kostic, K., Stojiljkovic, I., Salaj-Smic, E. & Trgovcevic, Z. (1992) *Mutat. Res.* **281,** 123–127.
43. Marsden, H. S., Pollard, E. C., Ginoza, W. & Randall, E. P. (1974) *J. Bacteriol.* **118,** 465–470.
44. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. (1983) *Cell* **33,** 25–35.