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Transcriptional and Mutational Analyses of the *Streptomyces lividans recX* Gene and Its Interference with RecA Activity

SILKE VIERLING, TILMANN WEBER, WOLFGANG WOHLLEBEN, AND GÜNTHER MUTH*

Mikrobiologie/Biotechnologie, Universität Tübingen, D-72076 Tübingen, Germany

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The role of the 20,922-Da RecX protein and its interference with RecA activity were analyzed in *Streptomyces lividans*. The *recX* gene is located 220 bp downstream of *recA*. Transcriptional analysis by reverse transcriptase PCR demonstrated that *recX* and *recA* constitute an operon. While *recA* was transcribed at a basal level even under noninducing conditions, a *recA-recX* cotranscript was only detectable after induction of *recA* following DNA damage. The *recA-recX* cotranscript was less abundant than the *recA* transcript alone. The *recX* gene was inactivated by gene replacement. The resulting mutant had a clearly diminished colony size, but was not impaired in recombination activity, genetic instability, and resistance against UV irradiation. Expression of an extra copy of the *S. lividans recA* gene under control of the thiostrepton-inducible *tipA* promoter was lethal to the *recX* mutant, demonstrating that RecX is required to overcome the toxic effects of *recA* overexpression. Since inactivation of the *recX* gene did not influence transcription of *recA*, the putative function of the RecX protein might be the downregulation of RecA activity by interaction with the RecA protein or filament.

RecA is a multifunctional protein that is involved in homologous recombination, DNA repair, and the induction of the SOS response (13, 35). The protein is highly conserved among all prokaryotes (12, 23), and homologues of RecA are also found in eukaryotes (2). Transcriptional regulation of *recA* by the SOS repressor LexA has been well studied in *Escherichia coli* and *Bacillus subtilis* (18, 36). Under normal growth conditions, the LexA protein binds to a specific DNA sequence, the SOS box, upstream of the promoter region and inhibits transcription. Following DNA damage, autocleavage of the LexA repressor results in the induction of the respective genes. The SOS box of gram-positive bacteria, GAAC-N4-GTTC/T, differs from the binding site for LexA, CTGT-N8-ACAG, in gram-negative organisms (6, 34).

In streptomycetes, the RecA protein is assumed to be involved in genetic instability, which is a remarkable feature of these mycelium-forming and antibiotic-producing bacteria. Their chromosome is highly unstable under laboratory conditions and can suffer from very large deletions at rates higher than 0.1% (33). Genetic instability affects different phenotypical properties, including morphological differentiation, production of secondary metabolites, such as pigments and antibiotics, antibiotic resistance, secretion of extracellular enzymes, and, sometimes, genes for primary metabolism. A plausible model for a specific role of RecA in ensuring viability has been suggested by Volff and Altenbuchner (32): the occurrence of single-stranded breaks within the chromosome might cause the replication fork to collapse, as was described for *E. coli* (14). Due to the linearity of the *Streptomyces* chromosome (15, 16), this would result in the loss of a chromosomal end, and mutants containing large deletions would be segregated. If the cell is recombination proficient, these breaks can be repaired and the chromosomal ends are rescued. In a completely recombination-deficient mutant, the high frequency of deletions might interfere with the viability of the cell.

In many organisms, a gene termed *recX* was identified downstream of *recA* (7). In mycobacteria, the *recX* gene overlaps with the coding region of *recA*, and the two genes are cotranscribed (24). Overexpression of the wild-type *recA* gene in a *Pseudomonas aeruginosa recA* mutant (*rec-2*) was only tolerated if the *recX* gene was simultaneously expressed. Therefore, a regulatory role for *recX* in RecA activity was suggested (26). However, it was not clear whether it controls the expression of the *recA* gene or interacts directly with the RecA protein (26).

Various attempts have been made to generate *recA* deletion mutants in streptomycetes. It was only possible to isolate disruption mutants with residual RecA activity (1, 21). Therefore, a crucial role of the *recA* gene in ensuring the viability of streptomycetes was suggested. However, it could not conclusively be excluded in these experiments that a polar effect on downstream genes (e.g., *recX*) was responsible for the failure to generate *recA*-deficient *Streptomyces lividans* mutants. Such polar effects on *recX* have also been discussed by Papavinasasundaram et al. (24) to explain the inability to inactivate *recA* of *Mycobacterium smegmatis*.

In this paper, we report the transcriptional analysis of the *S. lividans recX* gene and the construction of a *recX* gene replacement mutant. The phenotypic characterization of the mutant suggested that RecX downregulates RecA activity by protein-protein interaction to overcome the toxic effects of RecA overexpression.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The *E. coli* strain used for subcloning and DNA sequencing was XL1-Blue (4). The parental *Streptomyces* strain was *S. lividans* TK64. *E. coli* cells were grown at 37°C in Luria-Bertani (LB) medium (25). *Streptomyces* strains were cultured as described previously (8). Antibiotics were supplemented, where appropriate, at the following concentrations: ampicillin, 150 μg ml^{-1}; kanamycin, 50 μg ml^{-1}; thiostrepton, 25 μg ml^{-1}; gentamicin, 5 μg ml^{-1}; chloramphenicol, 10 μg ml^{-1}. The plasmids used in this work are listed in Table 1.

DNA manipulations. Standard procedures were performed as described by Hopwood et al. (8) and Sambrook et al. (25). Hybridization used digoxigenin-labeled dUTP and a digoxigenin detection kit (Boehringer, Mannheim, Germany). Gene replacement mutants were selected as described by Wohlleben and Muth (37).

Expression of *recX*. The *S. lividans recX* gene was amplified by PCR with primers 5recX and 3recX (Table 2). Following restriction with *Nde*I and *Bam*HI, the fragment was inserted into the *Streptomyces* expression vector pIJ4123 (30), yielding plasmid pSVX-his. In pSVX-his, the *recX* gene is expressed with an

* Corresponding author. Mailing address: Mikrobiologie/Biotechnologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany. Phone: 49 7071 2974637 or 49 7071 2974644. Fax: 49 7071 295979. E-mail: gmuth@biotech.uni-tuebingen.de.

TABLE 1. Plasmids used in this work

Plasmid	Description	Reference or source
pUC18	*lacZ bla*	31
pGM11	*aphII*; temperature-sensitive *Streptomyces* vector	37
pJF293.2	*bla*; P*tipA*	J. Altenbuchner, Stuttgart, Germany
pSVX1	*recX* replacement plasmid; pGM11 derivative carrying the 1,550-bp *Pst*I-*Nco*I fragment; *tsr* insertion within the *Bcl*I site	This study
pSVQ1	Recombination test plasmid; pGM11 derivative carrying a 1,316-bp *recQ* PCR fragment, disrupted by the *tsr* gene	This study
pSVX2	pGM11 derivative carrying the 1,685-bp *Sal*I fragment encoding RecX and the C-terminal half of RecA	This study
pSVAX2	*recX* complementation plasmid; pGM11 derivative carrying a 2,346-bp PCR fragment containing the complete *recA-recX* operon	This study
pIJ4123	*Streptomyces* His tag expression vector; *tsr kan* P*tipA redD*	30
pSVX-his	pIJ4123 derivative carrying a PCR fragment containing the *recX* gene	This study
p2001/41	Bifunctional SCP2 derivative, p15a *E. coli* ori, *tsr cat*	Unpublished data
pEXrecA	*recA* expression plasmid; p2001/41 derivative; P*tipA recA tsr aacC1*	This study
pEXR169-H	*recA* expression plasmid; p2001/41 derivative; P*tipA recA*(R169-H)	This study

N-terminal His tag under the control of the thiostrepton-inducible *tipA* promoter (P*tipA*) (20).

Preparation of *S. lividans* RNA. *S. lividans* was cultivated in 50 ml of YEME/LB (4+1) (YEME composition given in reference 8) for 2 to 3 days. The culture was induced with methyl methanesulfonate (MMS; 25 μg ml^{-1}) for 20, 40, and 60 min. Cells were harvested and shock frozen at −70°C. An aliquot was resuspended in 100 μl of P-buffer containing 0.33 mg of lysozyme and incubated for 7 min at 37°C. RNA was extracted from uninduced and MMS-induced cultures by using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

RT-PCR analysis. RNA prepared from *S. lividans* was treated with 3 U of RNase-free DNase I (Promega, Mannheim, Germany) and precipitated according to standard protocols (25). The RNA concentration was photometrically determined by using a Genequant fixed-wavelength photometer (Pharmacia, Freiburg, Germany). The reverse transcription reaction was carried out with an enhanced avian reverse transcriptase PCR (RT-PCR) kit (SIGMA, Germany) according to the manufacturer's instructions. A 5-μl aliquot of the RT reaction product was used as a template and amplified with *Taq* DNA polymerase (Qiagen). The PCR was carried out in a programmable Thermal Controller (MJ Research, Inc.) with the following profile: 1 cycle at 94°C for 120 s and 25 cycles at 94°C for 75 s, 60°C for 90 s, and 72°C for 110 s. The final step was an elongation reaction for 10 min at 72°C. The oligonucleotide primers are listed in Table 2. The PCR products were analyzed by agarose gel electrophoresis (1.0%).

Assay for UV sensitivity. Spore dilutions of the *recX* mutant and the corresponding parental strain were plated onto LB agar and irradiated with UV light (Vilber Lourmat, VL115c; 254 nm, 730 μW/cm^2) at a distance of 10 cm for various periods (2, 5, 10, 15, and 20 s), followed by incubation in the dark at 30°C for 3 days. Colonies were counted, and the percentage of survival was determined.

Assay for genetic instability. Chloramphenicol-resistant cultures from the wild type and the *recX* mutant were incubated for several sporulation cycles. Subsequently, serial dilutions of spores were plated on LB medium without antibiotic at 30°C for 3 days. To determine the frequency of chloramphenicol-sensitive cultures, 1,000 colonies from the wild type and the *recX* mutant, respectively, were picked in parallel on LB agar without and with chloramphenicol (10 μg ml^{-1}).

Assay for determining recombination activity. To analyze recombination activity, plasmid pSVQ1, a pGM11 derivative carrying an *S. lividans recQ* gene fragment disrupted by a thiostrepton resistance cassette, was used. In the *recX* mutant, *S. lividans* SVX1, pSVQ1 can integrate into the chromosome by homologous recombination between the *recQ* fragments (720 and 596 bp) or the thiostrepton resistance gene (1,060 bp). In *S. lividans* TK64, the plasmid can only integrate within the *recQ* fragment. pSVQ1 was transferred into *S. lividans* TK64 and into the mutant SVX1 by polyethylene glycol-mediated protoplast transformation. Equal amounts of transformants were inoculated in liquid culture for 1 day at 28°C and 3 days at 39°C to eliminate the temperature-sensitive plasmid. Subsequently, mycelium was homogenized, and serial dilutions were spread on kanamycin-containing plates and nonselective agar and incubated at 39°C. The titer on kanamycin-containing plates, which indicates plasmid integration in relation to the titer on nonselective agar, gives the integration frequency.

TABLE 2. Oligonucleotides used for RT-PCR analysis

Primer	Strand	Oligonucleotide sequence (5′→3′)
recA1	+	ATCGAGGTCATCCCGACCGGGTCT
recA2	−	ATGTCGATCAGACCGCCCTCGC
recA3	+	ATCAAGCAGAAGCTGGGCGTCGG
recX1	+	TCCTCGTCGAGGGCCGAGAAGG
recX2	−	CCGTGTCCTCGCCCTCTTCCTCC
glnA1	+	GGCGAGCAGTACTCCCGCGACC
glnA2	−	GTACACCAGGTTCACCGGCGCCTC
5recX	+	GGAATTCCATATGGAGCCGTCCGCCGAGGA
3recX	−	CGGGATCCTCGAGAACCCCTCGTCGCCGAGG

RESULTS

Identification of the *S. lividans recX* gene. At 220 bp downstream of the *recA* stop codon in *Streptomyces coelicolor* A(3)2 (EMBL accession no. AL020958), an open reading frame with significant similarity to the *recX* genes of *Mycobacterium leprae* (43.5% identity, 161-amino-acid [aa] overlap) and *P. aeruginosa* (28.5%, 147 aa) was identified. To prove the presence of the *recX* homologue and the conservation of the gene organization in *S. lividans*, we amplified the corresponding *S. lividans* fragment with primers deduced from the *S. coelicolor* sequence. Partial sequence analysis (data not shown) resulted in sequences identical to that of *S. coelicolor*. The *recA-recX* intergenic region contains several direct repeats and has the potential to form secondary structures. A hairpin structure with 20 bases in the stem and 7 in the loop ($\Delta E = -26.2$ kJ/mol) which could act as a transcriptional terminator of *recA* transcription is located 64 bp downstream of the *recA* stop codon. This putative termination structure is also present downstream of the *Streptomyces ambofaciens recA* gene (1).

***recX* is cotranscribed with *recA* after induction with the DNA-damaging agent MMS.** The distance of 220 bp between *recA* and *recX* and the putative termination structure downstream of *recA* suggested that these two genes were transcribed independently in *S. lividans*. An RT-PCR analysis was performed to assess whether both genes were cotranscribed. Since *recA* of *S. lividans* is regulated by the SOS repressor LexA (unpublished results), RNA was isolated after induction with the DNA-damaging agent MMS (11). Primer pairs within *recA* (recA1 and recA2) and *recX* (recX1 and recX2) were used to detect the independent transcription of each gene. In order to prove the presence of *recA-recX* cotranscripts, primers corresponding to the 3′ region of *recA* (recA3) and *recX* (recX2) were chosen (Table 2 and Fig. 1). The functionality of the primers for RT-PCR was demonstrated by PCR on genomic DNA as the template (Fig. 2, lane DNA). The absence of

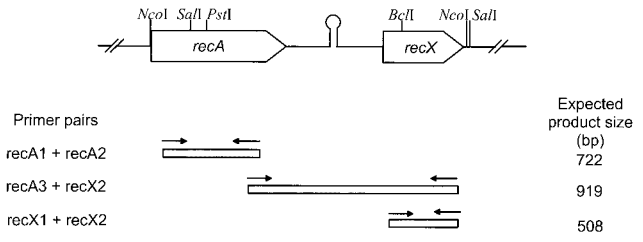

FIG. 1. Organization of the *S. lividans recA-recX* operon. *recA* and *recX* are separated by a 220-bp fragment containing a hairpin structure ($\Delta E = -26.2$ kJ/mol). Only relevant restriction sites are given. The primers used in the RT-PCR to identify *recA* and *recX* transcripts or *recA-recX* cotranscripts are indicated by arrows. The expected product sizes formed by the different primer combinations are listed on the right.

contaminating DNA in the RT-PCR was confirmed by a control PCR with RNA as a template (Fig. 2E). From uninduced cultures, no *recX* transcript and only a weak band indicating basal expression of the *recA* gene were detected (Fig. 2A to C, lane 1). Twenty minutes after induction with DNA-damaging MMS, the intensity of the *recA*-specific band increased, demonstrating induction of the *recA* gene during the SOS response. Transcription of the *recX* gene, however, was not detectable even 20 min after induction. A *recA-recX* cotranscript appeared only 40 and 60 min after induction (Fig. 2B, lanes 3 to 4), when expression of *recA* reached its maximum (Fig. 2A, lanes 3 and 4). This demonstrated that *recX* was cotranscribed with *recA* after induction of the SOS response. Probably due to the termination structure between *recA* and *recX*, the *recA-recX* cotranscript was produced only at a low level (less than 10%) compared to the *recA* transcript.

As a control for the quality of the RNA preparation, RT-PCR was also performed with a primer pair deduced from the *glnA* gene (glutamine synthetase I) which is not induced by DNA damage. In contrast to the *recA* and *recA-recX* message, the intensity of *glnA* transcription did not significantly change during MMS induction (Fig. 2D).

Construction of a *recX* gene replacement mutant in *S. lividans* TK64. To analyze the role of RecX, we intended to inactivate the *recX* gene. Therefore, the cloned *recX* gene was disrupted by the insertion of the thiostrepton resistance gene into the single *Bcl*I site located in the N-terminal half of *recX*. The temperature-sensitive replacement plasmid pSVX1 (Fig. 3A), which carries the disrupted *recX* gene, was transferred into *S. lividans* TK64, and colonies were selected with the thiostrepton resistance marker integrated into the chromosome. Subsequently, the colonies were picked on LB agar containing thiostrepton (25 μg ml^{-1}) or kanamycin (50 μg ml^{-1}). One out of 600 colonies was found to be thiostrepton resistant and kanamycin sensitive, indicating gene replacement and plasmid loss.

To verify the gene replacement, genomic DNA of the mutant was isolated and subjected to Southern blot analysis with a probe encoding the C-terminal part of RecA and the complete RecX. The probe hybridized with a 3,168-bp fragment that is 1,060 bp larger than the fragment in the wild type (Fig. 3B). This increase in size was due to the insertion of the *tsr* cassette. In addition, the *recX* genotype was further confirmed by PCR. With primers (recX1 and recX2) flanking the insertion site within *recX*, a fragment from the mutant was amplified which was 1,060 bp larger than the corresponding wild-type fragment (Fig. 3B).

Phenotype of the *S. lividans* SVX1 mutant. The *recX* mutant showed normal wild-type morphology on agar plates and in liquid culture. Only when spores were plated on solid medium was the colony size of the mutant clearly reduced (about 30% of the wild-type area) compared to that of *S. lividans* TK64 (Fig. 4). In order to investigate the effect on RecA-related functions, the UV sensitivity, the ability to undergo homologous recombination, and the genetic instability of the mutant SVX1 were analyzed.

As demonstrated in Fig. 5, the UV sensitivity of SVX1 was not significantly affected, indicating that the mutant is still proficient in recombinational DNA repair and able to induce the SOS response.

To test the recombination activity of the SVX1 mutant, the

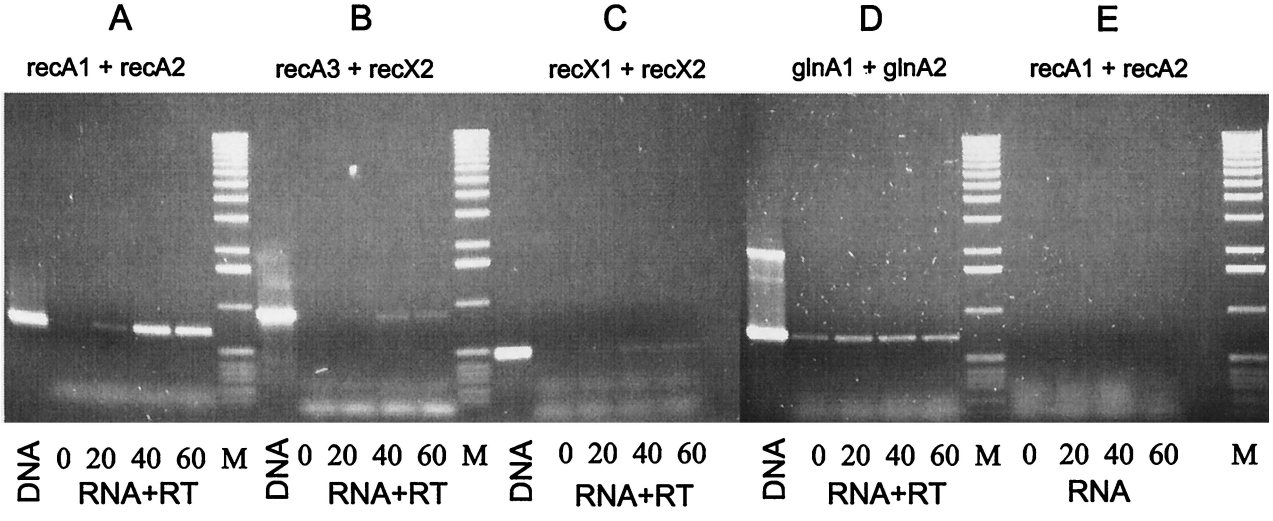

FIG. 2. Transcriptional analysis of the *recA-recX* operon by RT-PCR. After induction with 25 μg of MMS ml^{-1}, RNA was isolated from *S. lividans* TK64 at different intervals and used for RT-PCR with different primer combinations. (A) *recA*-specific primers. (B) *recA-recX* cotranscript-specific primers. (C) *recX*-specific primers. (D) Control reaction using *glnA*-specific primers. (E) Control reaction without RT. Lanes: DNA, control PCR with genomic DNA as template; 0, without induction; 20, 40, and 60, 20, 40, and 60 min after induction, respectively; M, size standard (1-kb ladder [Boehringer]: 12,216, 11,198, 10,180, 9,162, 8,144, 7,126, 6,108, 5,090, 4,072, 3,054, 2,036, 1,636, 1,018, 517, 506, 396, 344, 298, 220, 201, 154, 134, and 75 bp).

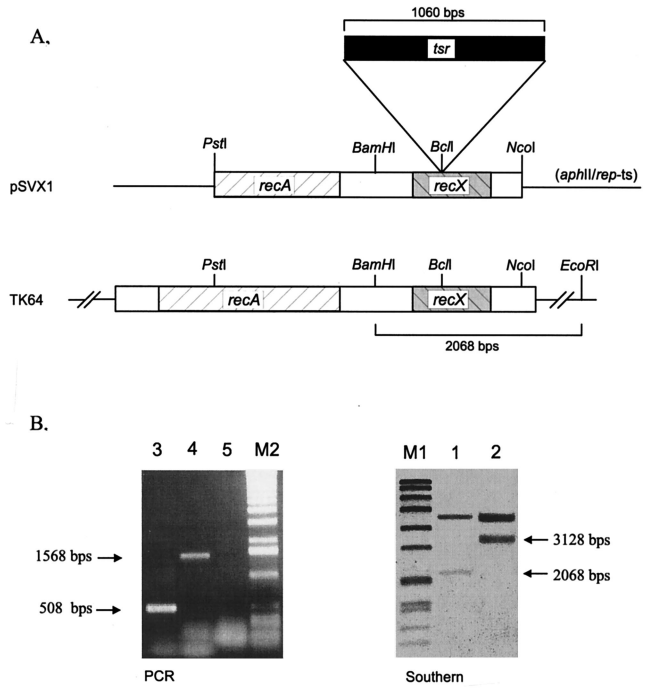

FIG. 3. Construction of an *S. lividans recX* mutant. (A) The *recX* gene was disrupted by the insertion of the thiostrepton resistance gene *tsr* into the single *Bcl*I site and cloned into the temperature-sensitive pGM11 plasmid, yielding pSVX1. The chromosomal *recX* copy was replaced via a double-crossover resulting in the mutant SVX1. (B) Using the 1,550-bp *Pst*I-*Nco*I fragment as a probe and *Bam*HI-*Eco*RI-digested total DNA of *S. lividans* TK64 (lane 1) and the mutant SVX1 (lane 2), the correct gene replacement was confirmed by Southern blotting. For PCR, the primers recX1 and recX2 (Fig. 1) which flank the *Bcl*I site (lane 3, TK64; lane 4, SVX1) were used. The absence of the replacement plasmid was demonstrated by using internal *aphII* primers (lane 5). M1, Bio-VII-Marker (Boehringer) (8,576, 7,427, 6,106, 4,899, 3,639, 2,799, 1,953, 1,882, 1,515, 1,482, 1,164, 992, 710, 492, and 359 bp). M2, 1-kb ladder (Boehringer) (12,216, 11,198, 10,180, 9,162, 8,144, 7,126, 6,108, 5,090, 4,072, 3,054, 2,036, 1,636, 1,018, 517, 506, 396, 344, 298, 220, 201, 154, 134, and 75 bp).

ability to integrate the temperature-sensitive plasmid pSVQ1 (Table 1) into the chromosome was studied as described in Materials and Methods. The plasmid integrated into the SVX1 chromosome with an efficiency of 2 to 10%, similar to that in the parental TK64 chromosome, demonstrating that the *recX* mutant pSVX1 is recombination proficient and that inactivation of *recX* does not significantly affect the recombination activity of *S. lividans*.

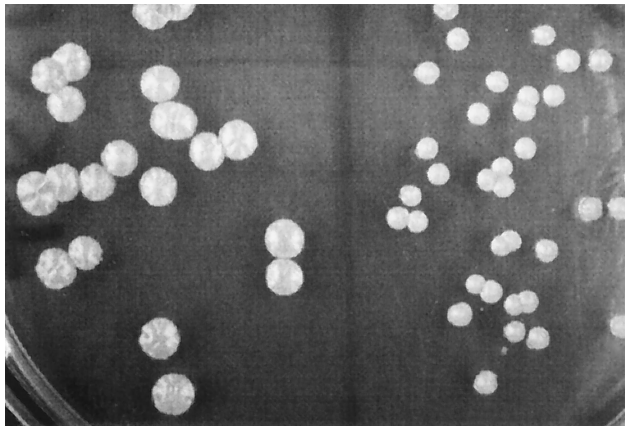

FIG. 4. Colony size of the *S. lividans recX* mutant SVX1 compared to that of TK64. Spores were plated on LB agar (left side, *S. lividans* TK64; right side, SVX1) and incubated at 30°C for 4 days.

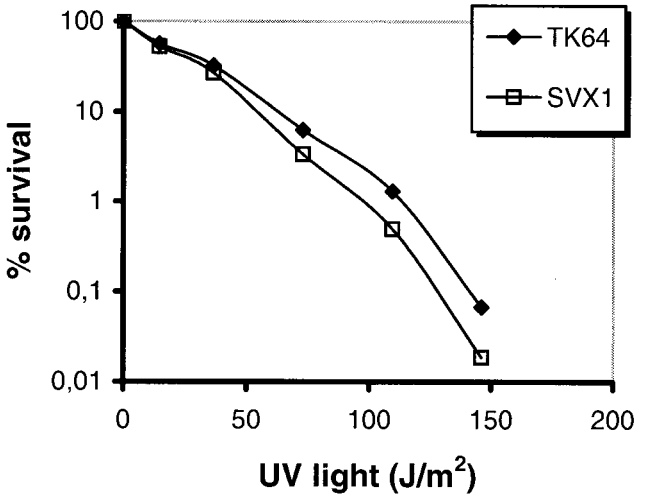

FIG. 5. UV sensitivity of the *S. lividans* SVX1 mutant. Spores of *S. lividans* TK64 and the *recX* mutant SVX1 were plated on LB agar and irradiated with UV light (254 nm, 730 $\mu W/cm^2$) for various periods.

The genetic instability of the *recX* mutant SVX1 was assessed by analyzing the segregation of chloramphenicol-sensitive colonies, which arise by the loss of the chromosomal end containing the chloramphenicol resistance gene (*cml*). The mutant SVX1 segregated chloramphenicol-sensitive mutants at wild-type frequency (0.8%), thus indicating that genetic instability was also not affected by the inactivation of *recX*.

The only visible effect of *recX* inactivation was the small colony size after plating spores on solid medium. To confirm that this phenotype was due to the inactivation of *recX* and was not caused by an additional mutation, it was necessary to show phenotypic reversal. The *S. lividans recX* gene was amplified by PCR and cloned into the high-copy expression vector pIJ4123 (30) under control of the thiostrepton-inducible *tipA* promoter (P*tipA*) (pSVX-his). On thiostrepton-containing agar, the wild-type colony size of SVX1 carrying pSVX-his was fully restored.

Mutant SVX1 could also be complemented by plasmid pSVAX2 containing the complete *recA-recX* operon, including the promoter region (Fig. 1). In contrast, plasmid pSVX2, which encoded the complete RecX and the C-terminal half of RecA but lacked a functional promoter sequence, was not sufficient to restore wild-type size.

Overexpression of RecA is toxic in the absence of RecX. To analyze the effects of *recA* overexpression in the *recX* mutant SVX1, the *recA* expression plasmid pEXrecA, an SCP2 (3) derivative which carried *recA* under control of the thiostrepton-inducible *tipA* promoter (20), was constructed. Transformants of *S. lividans* TK64(pEXrecA) and SVX1(pEXrecA) were grown for 2 days in liquid culture under uninduced conditions. Subsequently the cultures were homogenized, and the mycelial fragments were plated on medium containing thiostrepton (20 $\mu g\ ml^{-1}$) and gentamicin (5 $\mu g\ ml^{-1}$), respectively, to compare the colony titers under induced and noninduced conditions.

For *S. lividans* TK64(pEXrecA), the colony titer on thiostrepton was about 60% of that on gentamicin-containing plates, indicating the inhibitory effect of *recA* overexpression. For the *recX* mutant, however, no single colony could grow on thiostrepton-containing medium. This demonstrated that in-

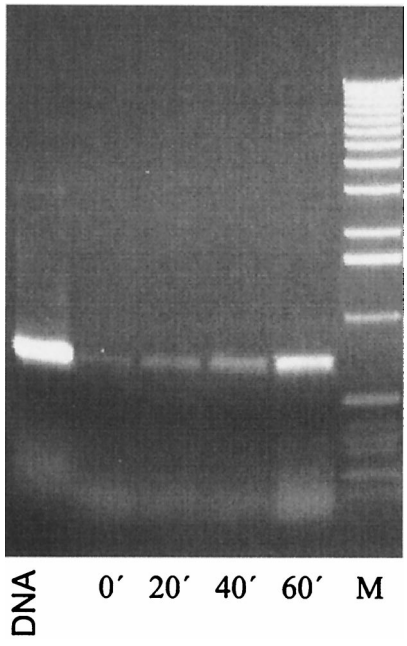

FIG. 6. *recA* transcription in the *recX* mutant SVX1. Following induction with MMS, the transcription of *recA* was analyzed by RT-PCR using primers recA1 and recA2 (Fig. 1). Lanes: DNA, control PCR using genomic DNA as a template; 0′, without induction; 20′, 40′, and 60′, 20, 40, and 60 min after induction, respectively; lane M, size standard (1-kb ladder [Boehringer]).

duction of the *tipA* promoter resulting in the overexpression of *recA* was lethal in the absence of RecX in *S. lividans*.

In contrast, if the *recX* gene was concomitantly expressed with *recA* in the *recX* mutant SVX1(pEXrecA pSVX-his), the colony titers under induced and noninduced conditions were the same (100%). Overexpression of *recX* allowed the cell to survive *recA* overexpression. This indicated that the function of RecX might be to counteract the toxicity of RecA.

To confirm that the toxic effects were caused by the biochemical activity of RecA and not by unspecific effects of protein overexpression, we expressed an inactive RecA (R-169H) protein in the mutant. In this mutant, arginine in position 169, which is one of the most conserved amino acid residues in bacterial RecA proteins and which is essential for the function (10), was replaced by a histidine residue (unpublished results). The inactive *recA*(R-169H) gene was inserted into the expression plasmid 2001/41 under control of the *tipA* promoter, and the resulting plasmid, pEXR169-H, was transferred into the mutant SVX1. After induction with thiostrepton, 95% of the titer compared to that under noninduced conditions was observed.

Transcription of *recA* is not influenced in the *recX* mutant SVX1. In order to analyze the mode of action of RecX, the influence of RecX on transcription of *recA* was investigated. RT-PCR with RNA isolated from the mutant SVX1 was performed. Only a faint band indicating the basal transcription of *recA* was detected without induction. Twenty minutes after administration of MMS, the intensity of the *recA*-specific band increased, and the maximum of *recA* transcription was reached after 60 min (Fig. 6). This clearly demonstrated that *recA* transcription is not significantly enhanced in the absence of RecX. Therefore, a role of RecX in repressing *recA* transcription is very unlikely.

DISCUSSION

The role of RecA in homologous recombination and in the induction of the SOS response has been elucidated in great detail (2, 13). However, only very little is known about the *recX* gene, which often is cotranscribed with *recA* and is supposed to be involved in modulating RecA activity (24, 26). By RT-PCR analysis, we showed that, as in other bacteria (7), the *S. lividans recX* gene is cotranscribed with *recA* following DNA damage, although the gene organization of the *Streptomyces recA* region suggested an independent transcription of *recX*. In contrast to *E. coli*, in which induction of the SOS response starts 1 min after UV irradiation and is completed after 4 to 5 min (28), expression of the *S. lividans recA* gene remained unchanged for the first 20 min. Only 40 min after induction, *recA* transcription reached its maximum. This delay in the induction of the SOS response in *Streptomyces* is difficult to understand. However, in *M. smegmatis* and *Mycobacterium tuberculosis*, the induction of the SOS response was also very slow, and the maximum levels of *recA* transcription were obtained 5 and 6 h after induction with DNA-damaging agents (19, 24). Simultaneously with the induction of *recA*, a *recA-recX* cotranscript appeared that was not detectable without induction. Two distinct transcripts are also formed in the *recA-recX* operon of *P. aeruginosa*. By Northern blotting, a 1.2-kb transcript representing the *recA* message and a 1.4-kb transcript comprising *recA* and *recX* were identified (9).

Although *recA* and *recX* form an operon in *S. lividans*, the transcription rates of both genes differ drastically. This is in contrast to *M. smegmatis*, in which both genes are transcribed with the same efficiency (24). Since the coding region of the *M. smegmatis recX* overlaps with the 3′ coding region of *recA*, it makes sense that the *recX* gene is always transcribed at the same level as *recA*. In *S. lividans* (and probably other *Streptomyces* strains), the weak termination structure between *recA* and *recX* might be responsible for transcription of *recA* without *recX* in the uninduced state and for the low level of *recA-recX* cotranscript in comparison to the level of *recA* transcript alone.

To elucidate the possible function of RecX, a *recX* mutant was constructed. Our ability to construct a *recX* mutant clearly showed that the *recX* gene is dispensable in *Streptomyces*. Therefore, the failure to inactivate *recA* (21) must be due to the *recA* gene itself and is not due to a polar effect on the downstream *recX* gene, as was discussed for *M. smegmatis* (24).

Only a very few data are available about the phenotypic effects of *recX* inactivation. The only published *recX* mutant represents a *recA recX* double mutant of *M. smegmatis* (24). Therefore, this mutant is not appropriate to analyze the function of RecX and its interference with RecA or RecA-related functions. For *P. aeruginosa*, a *recX* mutant was generated: in order to determine the coding region of the *P. aeruginosa recA* gene, several deletion mutants affecting not only *recA* but also the downstream regions were generated in the chromosome (9). As can now be deduced from the nucleotide sequence (26, 27), a *recX*-containing fragment has been deleted in mutant PDO7 *recAΔ34*. This deletion had only very slight effects on UV resistance. The recombination activity of PDO7 *recAΔ34* was not analyzed (9).

The *S. lividans recX* mutant SVX1 was not affected in any of the classical *recA*-related functions, but the small colony size in comparison to that of the wild type showed that RecX deficiency interferes with normal growth. A more drastic phenotype was observed when *recA* was overexpressed. Whereas induction of *recA* expression resulted in the reduction of the colony titer to about 60% in the wild type, indicating the toxic effect of *recA* overexpression, growth of the *recX* mutant was

completely inhibited. A similar observation was previously published by Sano (26) for *P. aeruginosa* and Papavinasasundaram et al. (24) for *M. smegmatis*. In these experiments, however, the authors intended to complement a *recA* (26) or a *recA recX* (24) double mutant and showed that *recA* could only be overexpressed if *recX* was coexpressed.

Since we could show that *recA* overexpression is lethal in a *recX* mutant, one would expect an impaired viability of the *recX* mutant after UV irradiation. DNA damage caused by UV irradiation should result in the induction of the SOS response and in overexpression of *recA* (17). Therefore, the *recX* mutant should not be inhibited directly by the UV irradiation, but due to the toxic action of RecA. However, the *recX* mutant was not significantly affected under these conditions. Probably, other SOS-induced genes are also involved in protecting the cell from RecA.

The nature of the toxic effect of *recA* overexpression is unknown. Since the *recX* mutant tolerated the overexpression of a mutated *recA* gene encoding an inactive protein, the toxic effects of RecA must be caused by one of the biochemical activities of RecA. The expression of several heterologous RecA proteins, e.g., from *P. aeruginosa*, *B. subtilis*, and *Deinococcus radiodurans*, and RAD51 from *Saccharomyces cerevisiae* has also been shown to be toxic to *E. coli*. In these cases, an enhanced affinity for DNA was suggested to be responsible for the toxicity (38). A mutant *E. coli* RecA(E-96D) protein that was toxic has been shown to prevent proper chromosome segregation (5).

Overexpression of *recA* was only tolerated in the mutant SVX1 when *recX* was simultanously highly expressed. In addition, the small colony size of the mutant was also complemented to the wild-type size. Obviously, the N-terminal 20-aa elongation containing the His tag that results from the *Streptomyces* expression vector pIJ4123 (30) did not substantially interfere with the activity of RecX.

About the mode of action of the RecX protein in controlling RecA activity, only speculations exist. Due to the basic character of the RecX proteins (pI value of about 9 to 11) and the weak similarity to resolvases, a possible function of the *P. aeruginosa* RecX as a transcriptional repressor of RecA has been discussed (7, 26). However, transcription of *recA* was not affected in the *S. lividans recX* mutant. Following induction with MMS, the transcription rate after 60 min was the same as in the wild type. This demonstrates that RecX does not repress transcription of *recA*. Furthermore, it was shown by immunoblotting that the same amount of RecA protein was produced in the *recX* mutant as in the wild type (unpublished results). A very similar result was described for *P. aeruginosa*. Deletion of the *recX*-containing DNA fragment also did not influence *recA* transcription or production of RecA protein in *P. aeruginosa* PDO7 *recAΔ34* (9). Because RecX does not affect expression of *recA*, we propose an interaction of RecX with the RecA protein. This interaction could result in the inhibition of RecA activity to accelerate the shutdown of the SOS response.

Recently, it was suggested by Zaitsev and Kowalczykowski (38) that the function of RecA proteins from distinct bacteria is adapted to the specific needs of a given organism by the modulation of monomer-monomer interaction strength. Since all of the biochemical functions of RecA are directly affected by the DNA binding, an alteration of the binding characteristics might efficiently modulate the specific activity of RecA. The RecX protein might be a candidate protein for controlling RecA. RecX could interact with the highly variable and species-specific C terminus (12) of RecA, which is located at the outer site of the RecA filaments (29), explaining why the RecX proteins from the different bacteria show only low sequence conservation (20 to 43% identity).

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