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Nuclease Overexpression Mutants of *Serratia marcescens*

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A family of mutants overexpressing the *Serratia marcescens* extracellular nuclease has been known for decades. A number of these alleles are characterized here at the molecular level, and the mutant genes are identified, yielding a likely model for their phenotype. The known mutations exert their effect indirectly on *nucA* expression by elevating the basal SOS response of the cell. Mutations have been found in *xerC* and *uvrD*, both of which result in partial SOS induction. A classic *nuc*^{su} allele, that of strain W1050, is also likely to be in *xerC*.

The production of extracellular nuclease is one of the defining attributes of *Serratia marcescens* and other *Serratia* species. However, nuclease is only one of a number of extracellular proteins expressed by species of *Serratia*; others include proteases, chitinases, lipases, and a bacteriocin called marcescin. Unlike these other secreted proteins, the extracellular nuclease does not appear to be subject to substrate or catabolite regulation. Nuclease is instead regulated by growth phase (4, 16), as is the synthesis of many other extracytoplasmic degradatory proteins, toxins, and bacteriocins (1, 8, 10, 11).

Many of the extracellular proteins of *Serratia* nevertheless share some common regulatory pathways with nuclease, and pleiotropic regulatory mutants that exert effects on nuclease, lipase, chitinase, and marcescin have been identified (4, 12, 17, 18). One such mutant, which abolishes nuclease production and reduces chitinase and lipase activity, has been shown to be in the *Serratia recA* gene (2). Consistent with SOS control, a LexA binding site is located in the nuclease gene *nucA* promoter region (4). SOS regulation has also been shown for other extracellular proteins, such as the pectin lyase of *Erwinia carotovora* (14). Other alleles abolishing nuclease as well as bacteriocin production define a novel regulatory locus, *nucC*, which is closely related to the *ogr* gene of temperate phage P2 (7, 13). The *nucC* operon is also growth phase and SOS regulated independently of *nucA*.

Yet another class of pleiotropic mutants overexpresses these proteins (4, 17, 18). These overexpression mutants (called *nuc*^{su} [17]) display no noticeable growth defect. They remain SOS inducible and are growth phase regulated. These alleles appear to fall into at least two categories based on their relative levels of nuclease overexpression. However, the nature of these mutations and the genes they represent have previously not been determined. The molecular characterization of these mutants is the focus of this work.

Mutant description. We previously described (4, 12) the isolation of transposon Tn*5* insertion mutations made in the wild-type *S. marcescens* strain SM6 that displayed altered nuclease halo sizes on DNase indicator plates. Among these were mutations that abolish expression of the nuclease (*nucA*) gene; these mutations map to the transcriptional activator gene *nucC* (13) as well as to *recA*. Other mutations displaying increased nuclease halo sizes were also described previously (4); these include mutants *nuc*^{su}93 and *nuc*^{su}95, which express nearly 50-fold more nuclease than SM6, and *nuc*^{su}161, which expresses about 10-fold as much nuclease as SM6. Similar mutants had previously been described, and one of these, W1050 (U. Winkler), which we use routinely, is derived from SM6(F′ *prolac*) and carries a chemically induced pleiotropic mutation causing the overexpression of nuclease as well as other extracellular enzymes (17, 18).

As a preliminary characterization, Southern blot analysis of the mutants was performed after digestion of mutant strain genomic DNA with *Eco*RI (*Eco*RI does not cut in Tn*5*) and probed with a radiolabeled 3.3-kb *Hin*dIII fragment from Tn*5*. This analysis (data not presented) showed that the insertions of *nuc*^{su}93 and *nuc*^{su}95 were on the same-size fragment of about 25 kb and that the *nuc*^{su}161 insertion was on a 9.5-kb fragment. The kanamycin resistance determinant of Tn*5* allowed easy cloning of fragments spanning Tn*5* from each of these mutants.

DNA sequence analysis. Sequence data obtained with a primer to the end of Tn*5* revealed that two of the mutants, *nuc*^{su}93 and *nuc*^{su}95, indeed have Tn*5* insertions in the same gene and at essentially the same site. This gene has significant sequence identity to *Escherichia coli xerC* (5). XerC is a subunit of the XerCD site-specific recombinase that serves to monomerize chromosomes and plasmids prior to cell division (3). The DNA flanking the insertion in *nuc*^{su}161 shows significant sequence identity to the *E. coli uvrD* gene. UvrD is DNA helicase II, which unwinds double-stranded DNA and is important in DNA replication, recombination, and repair (9). In *E. coli*, the *xerC* and *uvrD* genes are closely linked.

The entire region of interest was recovered as a single λ clone from a genomic SM6 λ library by probing the library with a fragment flanking the Tn*5* insertion from *nuc*^{su}161. Three fragments from this λ clone were subcloned, and from them, the nucleotide sequence of *xerC* and *uvrD* was determined from both strands by ABI Prizm DyeTerminator chemistry and run on the ABI 377 automated sequencer. The map of the region is shown in Fig. 1.

The DNA sequence is highly similar to the *E. coli* sequence (5) within the open reading frames but is highly divergent outside of the reading frames. There are four open reading frames found in this region, but for one of these (Orf235), we have the sequence for only the carboxyl terminus. XerC has 74% identity to *E. coli* XerC, Orf238 has 65% amino acid identity to *E. coli* Orf238, and UvrD has 88% identity to *E. coli* UvrD. The predicted proteins are identical in size, except that XerC has an additional four amino acids at its start and one amino acid at its end.

Measuring SOS induction. Why do Tn*5* insertions in *xerC* and *uvrD* lead to nuclease overexpression? The simplest explanation is that these mutants are partially induced for their SOS

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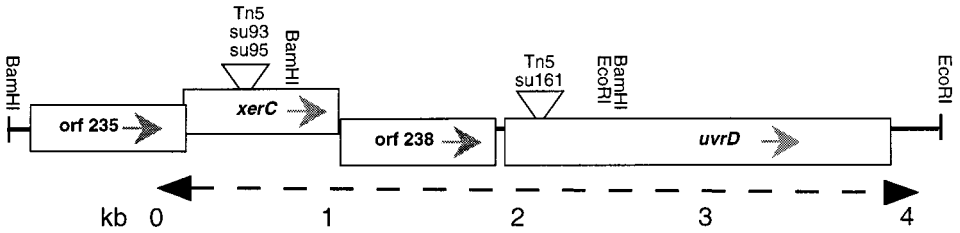

FIG. 1. Map of *S. marcescens xerC-uvrD* region. The gene order is identical to that of *E. coli*, and reading frames are oriented as indicated by the gray arrowheads. Sites of Tn*5* insertions are marked by inverted triangles; restriction sites are as shown. The region which has been fully sequenced is indicated by the dashed arrow with kilobase markers shown below the line.

response, resulting in induction of *nucA* transcription, since the *nucA* promoter is SOS inducible (4). *E. coli* strains with mutations in either *xerC* or *uvrD* show partial SOS induction (15a).

To test this, a *recA-lacZ* transcriptional fusion on the plasmid pMB684 was used to monitor SOS induction in the *S. marcescens* strains. Cultures were grown aerobically in Luria broth with 1 mg of ampicillin per ml at 30°C. In Table 1, the β-galactosidase activities of each strain harboring the tester plasmid are presented. All of the *nuc*^{su} mutants show a large increase in β-galactosidase activity compared to the wild-type SM6. W1050 is *lacZ*$^{+}$, which prevented us from accurately measuring SOS induction in this strain; however, its induction ratio is comparable to those of the strongest mutants (4). Another phenotype of SOS-induced cells is the formation of long filamentous cells. The *nuc*^{su} mutants and W1050 were all examined by phase-contrast light microscopy. All strains had a number of filamentous cells that were abnormally long compared to those of the SM6 control strain.

Suppression of the nuclease overexpression phenotype. Since *S. marcescens xerC* and *uvrD* have a high degree of similarity to their *E. coli* homologs, we tested whether the *E. coli* genes carried on multicopy plasmids could suppress the phenotype. Plasmid pSD105 carrying *E. coli xerC* and pET11d-H2wt carrying *E. coli uvrD* (9) were transformed into the different mutant and control strains. In Table 1, nuclease activity produced by these transformants is shown. SM6 containing these plasmids had no change in nuclease activity, whereas *nuc*^{su}93, *nuc*^{su}95, and W1050 containing pSD105 now produced wild-type levels of nuclease. Plasmid pET11d-H2wt had no effect on nuclease activity of strain W1050. From this, we conclude that the W1050 mutation is also in *xerC*. We were unable to obtain stable transformants of *nuc*^{su}93 or *nuc*^{su}95 with this plasmid. The *nuc*^{su}161 mutant containing pET11d-H2wt also produced wild-type nuclease levels, but pSD105 had no effect in this strain. These data show that the *E. coli* homologs specifically suppress the nuclease overexpression phenotype; therefore, the nuclease overexpression phenotype is in fact due to the Tn*5* insertion mutations and not to a secondary mutation elsewhere.

TABLE 1. Expression from the *recA* and *nucA* promoters

Strain[c]	β-Gal activity[a] of pMB684[d]	Nuclease activity[b]		
		No plasmid	pSD105[e]	pET11d-H2wt[f]
SM6	320 (10)	1	1	0.7
W1050	ND[g]	80	1	40
MB984*nuc*^{su}93	920 (40)	70	1	ND[g]
MB986*nuc*^{su}95	1,090 (50)	70	1	ND[g]
MB1454*nuc*^{su}161	820 (90)	30	30	0.8

[a] β-Galactosidase activity was measured according to the Miller (15) procedure, and the average of the activities from three independent stationary-phase cultures is shown along with the standard deviation in parentheses.

[b] Nuclease activity was measured from the cell-free supernatant of an overnight culture by the microtiter dish assay (2). The assay determines the dilution factor required to show no loss of fluorescence by using DNA in the presence of ethidium bromide as substrate. The values, normalized to those of the wild-type strain SM6, represent the averages of triplicate measurements.

[c] Wild type and *nuc*^{su} mutants containing plasmids were grown in Luria broth plus 1 mg of ampicillin per ml overnight.

[d] pMB684 Φ(*recA-trp'CBA lacZYA*).

[e] Plasmid pSD105 carries the *E. coli xerC* gene.

[f] Plasmid pET11d-H2wt carries the *E. coli uvrD* gene.

[g] ND, not done. Strain W1050 was already Lac^{+} and we were unable to transform strains MB984*nuc*^{su}93 and MB986*nuc*^{su}95.

Conclusions. In this work, we demonstrate that most nuclease overexpression mutants (*nuc*^{su}) are likely due to an indirect effect on transcription of *nucA* by the SOS system. The mutant phenotype is a result of partial induction of the SOS system caused by the mutation. Here we have sequenced two genes in which such mutations have been identified, *xerC* and *uvrD*. These genes are very similar to their *E. coli* homologs (5), and complementation data show that the *E. coli* genes are able to functionally replace the defective *S. marcescens* genes. The ability of *E. coli* XerC to repress the nuclease overexpression in W1050 suggests that it is also a *xerC* mutant.

We had previously suggested that these mutations may represent direct or indirect repressors of *nucA* expression (4, 13). Here we have demonstrated that this not correct. The *nucA* gene is regulated positively by the Ogr homolog NucC and negatively by LexA. Although growth phase clearly plays a role in the temporal regulation of nuclease production (16), likely due to accumulation of an extracellular factor (16a) like a homoserine lactone analog (6, 11), we do not know whether the regulatory factors responsible for this act directly at the *nucA* promoter, through NucC, or through the SOS system.

Nucleotide sequence accession number. The DNA sequence has been deposited in GenBank (accession no. AF028736).

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