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Involvement of σ^S in Starvation-Induced Transposition of *Pseudomonas putida* Transposon Tn*4652*

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Transpositional activity of mobile elements can be induced by different environmental stresses. Here, we present evidence that transposition of Tn*4652* is elevated in stationary-phase *Pseudomonas putida* and suppressed in an isogenic σ^S-defective strain. We demonstrate that transcription from the Tn*4652* transposase promoter is controlled by the stationary-phase-specific sigma factor σ^S. To our knowledge, this is the first example of direct stationary-phase-specific regulation of a mobile element transposase. Data presented in this report support the idea that activation of transposition under stressful conditions could be an inducible process.

Transposons are widespread in genomes and have important roles in evolution. Transpositional activity of a mobile element is generally maintained at a low level, yet a high frequency of transposition may occur in response to certain environmental stimuli. It has been shown that different stresses, such as carbon starvation (17), temperature effects (16, 21), and UV light (7), can enhance transposition of bacterial mobile elements. Moreover, it is hypothesized that activation of transposition under stress conditions might serve as an adaptive response to overcome stress and permit new traits to evolve (4, 24). However, the exact molecular mechanisms that underlie stress-induced transposition remain undefined.

Transposon Tn*4652* is a 17-kb-long deletion derivative of the toluene degradation transposon Tn*4651. Pseudomonas putida* strain PaW85 harbors Tn*4652* in the chromosome. Mutational processes in *P. putida* PaW85 have been previously studied in starving conditions on phenol minimal plates (13). That work showed the emergence of phenol-utilizing mutants due to the activation of transcription of plasmid-encoded promoterless phenol degradation genes *pheBA* in the plasmid pEST1414. About one-third of the starvation-induced Phe$^+$ mutants appeared as a result of insertion of Tn*4652* in front of the phenol monooxygenase gene *pheA* (13) (Fig. 1A). The transposition resulted in the formation of a fusion promoter between the transposon-inverted repeat and target DNA (13, 19). Interestingly, transposition of Tn*4652* seemed to depend upon the physiological state of bacteria: transposition frequency increased with time of starvation, whereas no Tn*4652*-linked Phe$^+$ mutants were detected among growing cells of *P. putida* (13). This indicated that starvation might increase transposition activity of Tn*4652*.

By the adaptation of bacteria to limited nutrient availability, changes in gene regulation take place, i.e., several genes are shut down while others are induced. One of the upregulated genes, *rpoS*, codes for an alternative sigma factor, σ^S, which controls expression of multiple stationary-phase genes (10, 18). In order to examine the potential role of σ^S in the regulation of Tn*4652*, we measured transposition of Tn*4652* in the wild-type *P. putida* PaW85 and in an isogenic σ^S-defective strain.

Transposition of Tn*4652* is decreased in the *P. putida rpoS* mutant strain. Transposition of native Tn*4652* was examined in a starvation assay as described previously (13), except that target plasmid pEST1332 was used instead of pEST1414. Similar to pEST1414, plasmid pEST1332 (15) contains the promoterless *pheBA* operon. However, it is more suitable for probing transposition of Tn*4652* since most of the Phe$^+$ clones arising on phenol minimal plates emerge from the insertion of Tn*4652* (19). Plasmid pEST1332 contains a specific target site that is preferred over the other sites present in both pEST1332 and pEST1414. To study the effects of σ^S on transposition of Tn*4652*, plasmid pEST1332 was introduced into *P. putida* PaW85 and into its *rpoS*-defective derivative PKS54. Bacteria were grown overnight (ON) in Luria-Bertani medium at 30°C and washed with M9 solution. Approximately 10^9 cells of ON cultures of PaW85 and PKS54 were spread onto five phenol minimal plates, and the accumulation of mutant Phe$^+$ colonies was monitored upon incubation of the plates at 30°C for 7 days. Results presented in Fig. 1B demonstrate that the emergence of Phe$^+$ mutants in the *rpoS*-defective *P. putida* was strongly suppressed. Appearance of Phe$^+$ mutants in the *rpoS*-defective strain was reduced 5 to 10 times compared to that in the wild-type *P. putida*. In order to test the insertions of transposon Tn*4652* into pEST1332, Phe$^+$ mutants were analyzed by PCR with oligonucleotides pheA, TnR, and TnL (Table 1). PCR analysis of Phe$^+$ colonies of the *P. putida* wild-type strain revealed that more than 95% of these mutants contained a Tn*4652* insertion upstream of the *pheA* coding region. In contrast, only about 20 to 30% of the Phe$^+$ colonies that appeared in the *P. putida* σ^S-defective strain carried a Tn*4652* insertion in pEST1332 (Fig. 1B). Thus, the absence of σ^S protein decreased transposition substantially, by more than 1 order of magnitude, but did not prevent it entirely. Here, we want to point out that the Phe$^+$ colonies revealed similar patterns of insertions in both the wild-type and *rpoS*-defective strains. Also, previous results indicate that RpoS is not obligatory for

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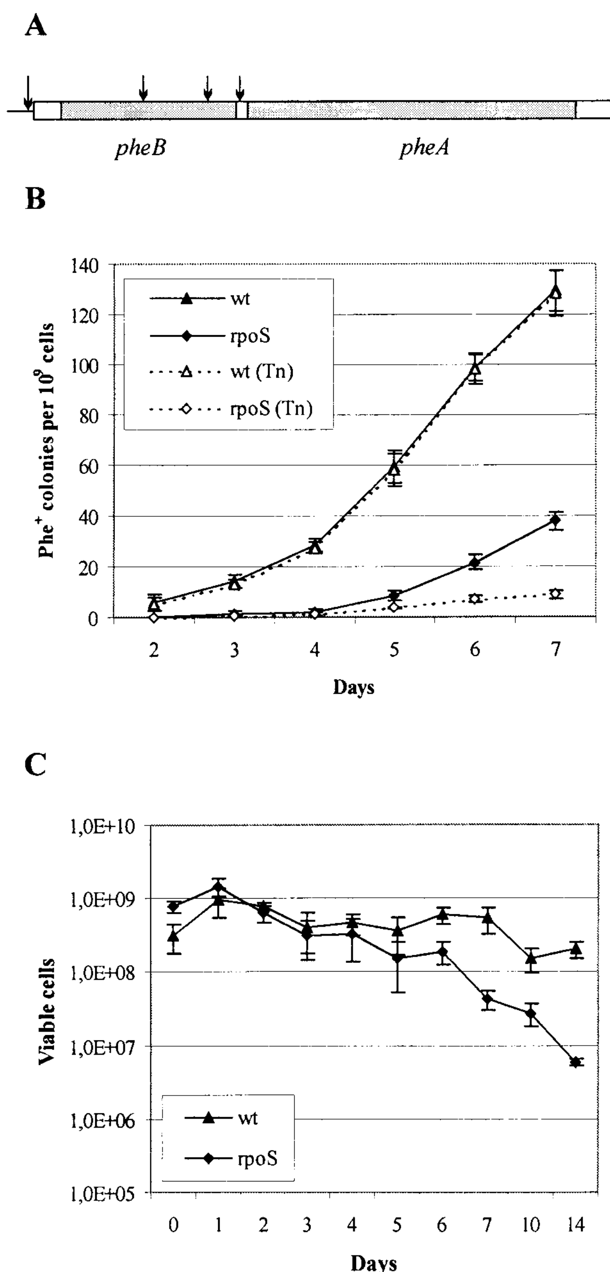

FIG. 1. (A) Schematic presentation of transposition target region in plasmid pEST1332. Catechol 1,2-dioxygenase (*pheB*) and phenol monooxygenase (*pheA*) genes are indicated by grey boxes. Vector DNA of pAYC32 is depicted with a line. Different insertion sites of Tn*4652* are indicated with arrows. (B) Accumulation of Phe^+ mutants on phenol minimal plates is indicated for *P. putida* strain PaW85 (wt) and isogenic *rpoS*-defective strain PKS54 (rpoS) containing plasmid pEST1332. Each point represents the mean of five independent determinations, and error bars represent standard deviations. Dashed lines indicate the theoretical appearance of Tn*4652*-linked Phe^+ mutants deduced from the results of PCR analysis of Phe^+ colonies. Up to 30 Phe^+ mutants were subjected to analysis on each day. (C) Viability of *P. putida* PaW85 (wt) and PKS54 (rpoS) carrying plasmid pAYC32 on phenol minimal plates. Each point represents the mean of five independent measurements, and error bars represent standard deviations. 1,0E + 08, e.g., marks 10^8 viable cells.

transcription from the fusion promoters created by Tn*4652* insertions (20).

RpoS is known to contribute to the maintenance of bacterial cell viability during the stationary phase of growth and during nutrient starvation (18, 22). Survival of *rpoS*-defective *P. putida* strain KT2440 has been demonstrated to decrease by 2 orders of magnitude during 1 week in liquid minimal medium (22; our unpublished results). Therefore, we estimated the viability of starving *P. putida* PaW85 and PKS54 on phenol minimal plates. In this experiment, *P. putida* PaW85 and PKS54 carrying plasmid pAYC32 (which differs from pEST1332 by its lack of the *pheBA* genes) were used in order to avoid the accumulation of Phe^+ mutants. About 5×10^8 to 8×10^8 bacteria of PaW85(pAYC32) and PKS54(pAYC32) were plated onto five phenol minimal plates, and small plugs were cut from the agar on each starvation day. Bacteria from the plugs were suspended in M9 solution, and the number of colony-forming units was determined on glucose minimal plates supplied with carbenicillin. Data in Fig. 1C show that viability of the σ^S-defective strain decreases slowly during 14 days of starvation on phenol plates; by the end of the second week, the number of viable cells of PKS54(pAYC32) had decreased by 2 orders of magnitude. However, during the first 6 days of starvation, survival of the σ^S-defective strain dropped only twofold. This cannot explain how Tn*4652*-linked Phe^+ mutants had an accumulation rate in PKS54(pEST1332) that was more than 10-fold lower than that in PaW85(pEST1332) (Fig. 1B). Therefore, we conclude that σ^S can act as a positive regulator in transposition of Tn*4652*.

Expression of transposase of Tn*4652* is σ^S dependent. How can RpoS control transposition of Tn*4652*? Transposition is mostly regulated by the amount and activity of transposase, the protein that performs the transposition reaction. Therefore, we evaluated the amount of transposase (TnpA) of Tn*4652* in a σ^S-defective background by Western blot analysis with an anti-TnpA polyclonal antiserum. TnpA is downregulated by the Tn*4652*-encoded TnpC, and therefore, the concentration of TnpA in the Tn*4652* background is not detectable by Western blot analysis (12). Yet, TnpA protein can be shown by this method if the copy number of the *tnpA* gene is increased by cloning the *tnpA* into plasmid pKT240 [plasmid pKTtnpA(D/H)] (12). Thus, we performed Western blot analysis with cell lysates prepared from ON cultures of *P. putida* PaW85 and PKS54 carrying plasmid pKTtnpA(D/H). We found that expression of plasmid-encoded TnpA was substantially decreased in the σ^S-defective strain; no TnpA protein could be detected by Western blot analysis in PKS54 (Fig. 2).

Transcription from the transposase *tnpA* promoter of Tn*4652* is growth phase controlled and σ^S dependent. In order to test whether the Tn*4652*-encoded transposase could be under the control of σ^S, the transcriptional activity of the *tnpA* promoter (Fig. 3A) was examined in *P. putida* strains PaW85 and PKS54. Previously, transcriptional fusions of the *tnpA* promoter region with the reporter gene *lacZ* have been constructed, and it has been demonstrated that the *tnpA* promoter is positively affected by integration host factor (IHF) (11). It has been shown that σ^S is involved in the regulation of the expression of IHF in *Escherichia coli* (1). Therefore, the *tnpA* promoter constructs either containing or lacking the IHF binding site (plasmids pKTlacZS/C and pKTlacZD/C, respectively)

TABLE 1. Bacterial strains, plasmids, and oligonucleotides

Strain, plasmid, or oligonucleotide	Description	Reference
Strains		
P. putida		
PaW85	Tn*4652*	3
PKS54	Tn*4652 rpoS*::Km	20
Plasmids		
pAYC32	Broad-host-range vector (Ap^r)	6
pEST1332	Plasmid pAYC32 carrying promoterless *pheBA* operon	15
pKT240	Cloning vector (Ap^r Km^r)	2
pKTtnpA(D/H)	Tn*4652 tnpA* gene cloned into pKT240	12
pKTlacZS/C	122-bp Tn*4652 tnpA* promoter region with IHF binding site cloned into pKTlacZ	11
pKTlacZD/C	65-bp Tn*4652 tnpA* promoter region lacking IHF binding site cloned into pKTlacZ	11
Oligonucleotides		
pheA	5′-TGCTCAAGATTATCATTACGCT-3′ (positions 11–32 in the *pheA* coding region)	
TnR	5′-ATCAGCATAGACGGCTAGCCAG-3′ (positions 101–122 from Tn*4652* right end)	
TnL	5′-CTTCCTCAATGGATGGCTGAAG-3′ (positions 111–132 from Tn*4652* left end)	

were tested in the σ^S-defective background. The results presented in Fig. 3B demonstrate that the transcription from the *tnpA* promoter is entirely dependent on the growth phase of the bacteria. Both reporter plasmids, pKTlacZS/C and pKTlacZD/C, tested in PaW85 exhibited stationary-phase-specific induction of the *tnpA* promoter. Also, as demonstrated previously (11), an about five- to sixfold-higher positive effect became apparent in the presence of the IHF binding site upstream of the *tnpA* promoter (Fig. 3B). Measurement of the β-galactosidase expression in the σ^S-defective *P. putida* strain PKS54 revealed that no obvious increase could be detected with either pKTlacZS/C or pKTlacZD/C during growth (Fig. 3B). Bacteria harboring either plasmid pKTlacZS/C or pKTlacZD/C showed similar and only slightly detectable levels of β-galactosidase activity that remained 50- or 10-fold lower, respectively, than that estimated in the wild-type strain, and no positive effects of the IHF binding site could be detected. Thus, these data indicate that stationary-phase-specific activation of the *tnpA* promoter specifically requires σ^S.

RpoS may act either directly on the *tnpA* promoter or indirectly by activation of some transcription factor operating on the *tnpA* promoter. Although σ^S- and σ^{70}-dependent promoters are generally quite similar, some subtle but essential differences in promoter sequences exist to ensure the selectivity between these two major sigma factors. σ^S-dependent promoters harbor mostly the sequence CTATACT in the conserved −10 region (8), while σ^{70} preferentially recognizes promoters with the sequence TATAAT. The −10 region CTATGCT of the *tnpA* promoter of Tn*4652* contains the sequence determinants suggested to be important for σ^S-dependent transcription, the C nucleotide upstream of the −10 hexamer and the C at the fifth position in the −10 hexamer (Fig. 3A). Therefore, we suppose that RpoS recognizes the *tnpA* promoter and is directly involved in the stationary-phase-specific expression of TnpA.

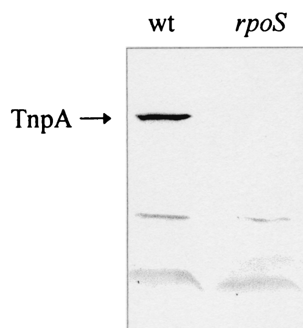

FIG. 2. Western immunoblot analyses of Tn*4652* TnpA in *P. putida* strain PaW85 (wt) and *rpoS*-defective strain PKS54 (*rpoS*) containing TnpA-expressing plasmid pKTtnpA(D/H). About 40 μg of crude cell lysate was loaded per lane.

Up to now the role of σ^S in regulation of transposition has been studied only in experiments with the mutant bacteriophage Mu. It has been shown that carbon starvation conditions trigger induction of mutant Mu prophage, resulting in formation of the *araB-lacZ* coding sequence fusions (17). Appearance of the *araB-lacZ* fusion clones on lactose-selective plates was completely abolished in a σ^S-negative *E. coli* strain (9). Since the transposase promoter of Mu was demonstrated to be not under the direct control of σ^S, it was supposed that σ^S could regulate Mu activation indirectly (17). Thus, according to our knowledge, σ^S-dependent upregulation of the transposase of Tn*4652* is the first example of direct stationary-phase-specific regulation of a mobile element transposase.

It is well known that plenty of mutations and other types of genetic variation are associated with the activity of mobile elements. Transpositional activity of most mobile elements is greatly suppressed, yet there are several examples of transposons that are activated under the conditions in which fast genetic changes are needed, i.e., under different stresses (5, 14, 23). An interesting question arises: does the activation occur due to malfunction of host defense mechanisms or is this an induced process to promote mutations that may potentially contribute to survival in unfavorable conditions? According to the results presented in this report, we prefer the latter version. Our results demonstrate that transposition of Tn*4652* is regulated by physiological conditions of the host. In starving bacteria, transposition of Tn*4652* is elevated due to direct control

A

GGGGTTATGCCGAGATAAGGCAAAAATTAGGACATTCGTTCTGTAAA

*Dra*I

TATATGATTTAAAAGGTTATTCGAGAGGCCGTGGCTTGCTGGTCATCC

*

ACCGTCTGGCTAGCCGTCTATGCTGATGCTTTTGCCTCGCTTGGGGG

B

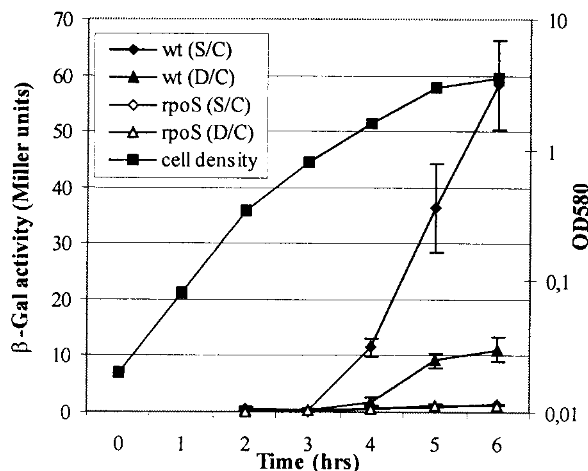

FIG. 3. (A) Sequence of right end of Tn*4652* containing promoter region of *tnpA*. The 46-bp inverted repeat is in boldface italics. The −10 hexamer of the *tnpA* promoter is boxed, and the transcription start of *tnpA* (11) is indicated by an asterisk. The potential IHF binding site is underlined. (B) Growth-dependent expression of *tnpA* promoter. *P. putida* wild-type strain PaW85 (wt) and its *rpoS* mutant PKS54 (rpoS) carrying either pKTlacZS/C or pKTlacZD/C were grown on Luria-Bertani medium. Plasmid pKTlacZD/C lacks the 57 nucleotides (up to the *Dra*I restriction site; for details, see the description for panel A) of the Tn*4652* right end sequence. Data (mean ± standard deviation) of at least four independent experiments are presented. OD580, optical density at 580 nm.

of the stationary-phase sigma factor σ^S that is induced just for better survival of cells in stressed conditions. Therefore, we believe that Tn*4652* serves as a good example of transposons that are activated under stressful conditions to increase the overall mutation rate and to generate new and potentially useful mutations.

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