JOURNAL OF BACTERIOLOGY, Nov. 1990, p. 6599–6601
0021-9193/90/116599-03$02.00/0

Vol. 172, No. 11

Molecular Analysis of an IS*200* Insertion in the *gpt* Gene of *Salmonella typhimurium* LT2

CATHERINE O'REILLY,[1]* GARY W. BLACK,[1] ROSEMARIE LAFFEY,[1] AND DAVID J. McCONNELL[2]

School of Biology, Sunderland Polytechnic, Sunderland SR1 3SD, United Kingdom,[1] and Department of Genetics, Trinity College, Dublin 2, Ireland[2]

Received 23 April 1990/Accepted 9 August 1990

A strain of *Salmonella typimurium* LT2 has been isolated which carries an insertion of approximately 700 bp in the *gpt* gene. The insertion in the *gpt* gene was shown to be the *Salmonella*-specific element IS*200*. The mutation in strain CR1 arose without selection during storage and is only the second phenotypically identified mutation caused by the insertion of IS*200*.

This article describes the isolation of a mutant, strain CR1, which contains an insertion in the *gpt* gene of *Salmonella typhimurium* LT2. This mutant arose without selection during routine subculturing of our stocks. The mutant strain (CR1) was identified as an 8-azaguanine-resistant derivative of the wild-type stock, strain WT1 (Table 1). Resistance to 8-azaguanine is normally the result of a mutation in the *gpt* gene, which encodes the enzyme guanine-xanthine phosphoribosyltransferase. A molecular clone of the *S. typhimurium* *gpt* gene was available (7), and with this plasmid, pSG7, as a probe, Southern blot hybridization of mutant and wild-type DNA was carried out. The results of this experiment are shown in Fig. 1. The wild-type *gpt* gene was carried on a 3.4-kb *Pst*I fragment (7), while the mutant gene was carried on a 4.1-kb fragment.

A detailed restriction map of a 20-kb region covering the *gpt proBA* region of wild-type *S. typhimurium* LT2 had previously been constructed (8; C. O'Reilly, Ph.D. thesis, Trinity College, Dublin, Ireland, 1983). By using a number of probes for this region in Southern blot hybridizations of the mutant CR1 strain, a restriction map of the homologous region was constructed (Fig. 1). From these maps, it is apparent that in the *gpt-83* mutant strain CR1 differed from the wild type by the insertion of a fragment of approximately 700 bp into the 3.4-kb *Pst*I fragment. The restriction maps of the wild-type and mutant strains were identical over the remainder of the 20-kb region which has been mapped. The 700-bp insertion contained additional restriction sites for *Eco*RI and *Hin*dIII. The presence and position of these sites, together with the absence of *Hin*cII sites in the inserted fragment, indicated that the insert in *gpt-83* may be the *Salmonella*-specific insertion sequence IS*200* (2–4).

Molecular cloning of the *gpt-83* mutation. To facilitate further analysis, molecular clones of the mutant gene were isolated. A *Hin*dIII gene library of the *gpt-83* mutant was constructed by using the lambda insertion vector NM1149 and screened with plasmid pSG7. As there is a *Hin*dIII site within the insertion (Fig. 1), two clones, phages CR6 and CR3, containing the left and right portions of the insertion, respectively, were isolated. The small *Hin*dIII-*Eco*RI fragment, which is an internal fragment of the insertion element, was further subcloned to give pRL1 (Fig. 1).

TABLE 1. Strains, phages, and plasmids used in this study

Strain, phage, or plasmid	Relevant	Source or reference
S. typhimurium LT2		
WT1	Wild type	Sunderland Polytechnic
WT2	Wild type	Trinity College, Dublin
CR1	*gpt-83*::IS*200*	Derived from WT1
Phages		
λCR6	9.4-kb *Hin*dIII fragment from the *gpt-83*::IS*200* locus cloned in λNM1149	This work
λCR3	3.4-kb *Hin*dIII fragment from the *gpt-83*::IS*200* locus cloned in λNM1149	This work
Plasmids		
pRL1	0.3-kb *Hin*dIII-*Eco*RI fragment from the *gpt-83*::IS*200* locus cloned in pUC18	This work
pSG7	Clone of wild-type *gpt* gene	8

Copy number analysis with pRL1. In order to measure the copy number of the insertion responsible for the *gpt-83* mutation, pRL1 was used as a probe in Southern blot hybridizations with restriction digests of strain CR1 DNA and DNA from two wild-type stocks (WT1 and WT2) of *S. typhimurium* LT2. WT1 is the stock from which the CR1 mutant strain was derived. The results (Fig. 2) indicate that WT2 contained at least five copies of the insertion element, while WT1 contained at least five copies of the insertion element, while WT1 contained at least seven copies and CR1 contained at least eight copies. Three differences were seen between WT1 and the CR1 mutant. WT1 contained a copy of the element which was absent in CR1, while CR1 contained two copies which were not present in WT2. One of these

* Corresponding author.

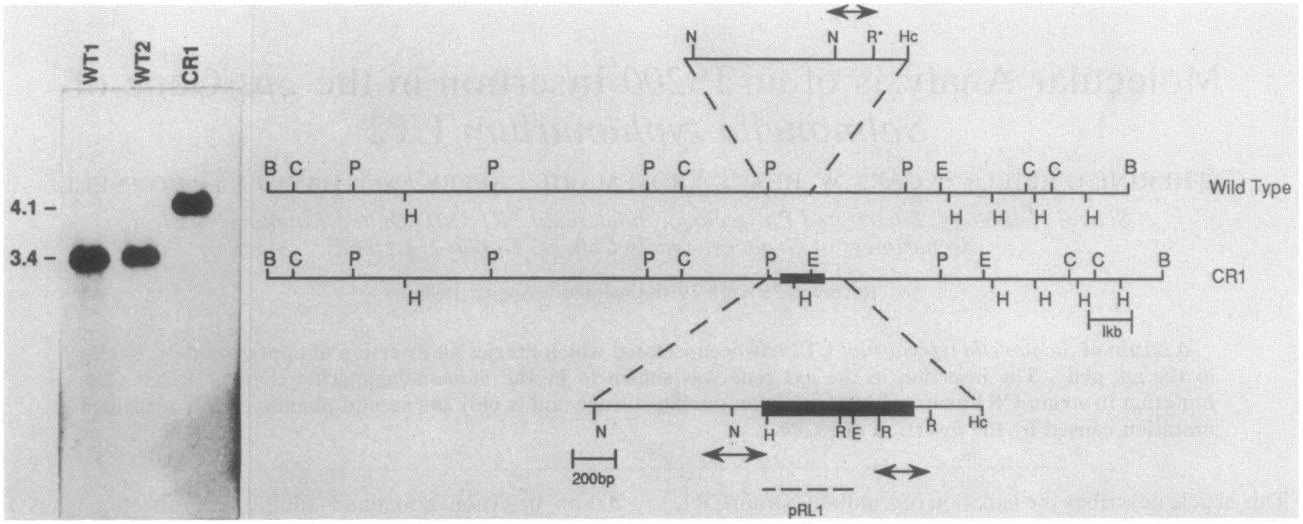

FIG. 1. Restriction analysis of the *gpt-83* mutation. (Left) DNA isolated from two wild-type strains of *S. typhimurium* and the CR1 mutant was digested with *Pst*I and, following Southern transfer, hybridized with pSG7. pSG7 contains a 3.4-kb *Pst*I fragment carrying the wild-type *gpt* gene. Sizes are shown in kilobases. (Right) Restriction maps of the *gpt proBA* region of the wild-type and CR1 mutant strains of *S. typhimurium*. Restriction enzymes: B, *Bam*HI; C, *Cla*I; P, *Pst*I; E, *Eco*RI; H, *Hin*dIII; N, *Nru*I; R, *Rsa*I; Hc, *Hin*cII. R* indicates that only this *Rsa*I site was mapped within this region. The position of the insertion in strain CR1 is indicated by the thicker line. The internal *Hin*dIII-*Eco*RI fragment subcloned in pRL1 is indicated, and the regions sequenced by subcloning into M13mp18 and M13mp19 are shown by arrows.

represents the insertion present in the *gpt* gene in this strain, while the other may represent a transposition event which occurred at the same time or subsequent to this transposition. The extra copy in the WT1 strain was unexpected but may be explained by a transposition event occurring subsequent to the separation of the two strains.

DNA sequence analysis of the IS element. DNA sequencing of the junction regions and the point of insertion was done by the dideoxy method in M13mp18 and M13mp19 (9). The regions sequenced are indicated in Fig. 1, and the sequencing data obtained are shown in Fig. 3.

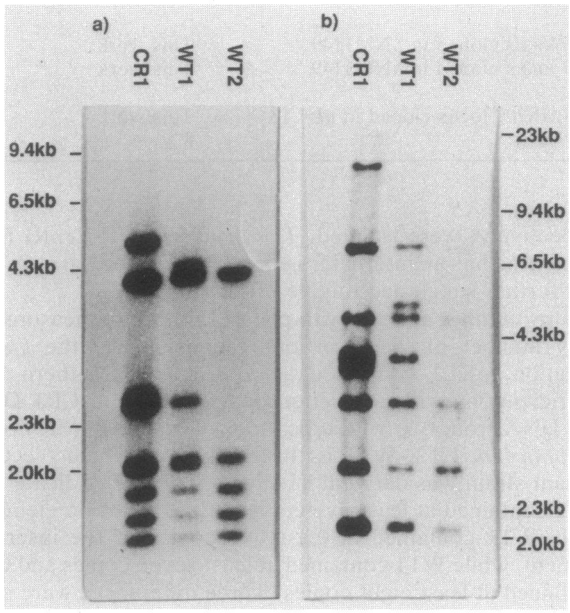

FIG. 2. Copy number analysis of IS*200*. Southern blot hybridizations of wild-type and CR1 mutant strains with the pRL1 probe. The DNAs were digested with (a) *Hin*cII and (b) *Pst*I.

A comparison of the sequence of the insertion with the published sequence of IS*200* (4) is shown in Fig. 4. A number of differences were seen between the two sequences, but the overall homology confirmed that the insertion in *gpt-83* was indeed IS*200*. The sequencing of the *gpt-83*::IS*200* allele supported the results of the analysis of the *his-984*::IS*200* allele (4), which indicated that IS*200* does not have terminal inverted repeats. Lam and Roth (4) had difficulty in defining the ends of IS*200* precisely, as the element in *hisD* had inserted into a stretch of five A's. The sequence data indicate that insertion has generated at most a 2-bp target site duplication, but it is also possible that these 2 bp are part of the IS*200* element. Similar difficulties were seen with the *gpt-83* mutation, where insertion again has taken place in a run of A · T pairs, and again there was a possible target site duplication of 2 bp. By comparison with the *his-984*::IS*200*, the conclusion can be drawn that regardless of whether the two T's are assigned to the element or the target site, in both mutations the IS*200* element in the *gpt-83* mutation was 3 bp longer than the element in the *his-984* mutation.

The CR1 strain contained eight copies of IS*200*, two of which were not present in the parent strain WT1, while WT1 contained one copy which was not present in CR1. Both CR1 and WT1 had one copy in common which was not present in WT2. There are therefore three copies of IS*200* available for which the preinsertional target site is also available for analysis. Sequencing of these target sites together with the insertion junction sequences may reveal sequence homologies required for insertion and would also indicate whether the sequence heterogeneity seen at the ends of the two copies of IS*200* studied to date is common to other copies of the element. Matsutani et al. (6) suggested that IS*200* is related to the IS*630* element of *Shigella sonnei*, which is 1,164 bp in size and has terminal inverted repeats which show some sequence homology to one end of IS*200*. They

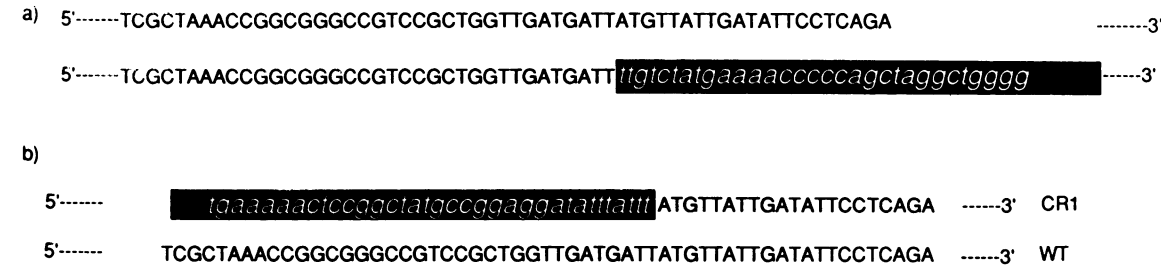

FIG. 3. DNA sequence analysis of *gpt-83*. Sequence analysis of the wild-type (WT) *gpt* gene and the left (a) and right (b) junction points of the insertion in the *gpt-83* mutation. The sequence of the insertion element is in the reversed-out area in lowercase letters.

speculate that the IS*200* found at the *hisD* locus (6) is in fact a deletion derivative of an IS*630*-like element and that the deletion occurred following insertion into the *hisD* gene. The results presented here do not support this hypothesis, as the element found in the *gpt* gene in CR1 appears to be almost identical in size to the element found in *hisD* and the sequences of the ends of the insertions in the two loci are almost identical. IS*200* appears therefore to be an unusual type of insertion element, capable of transposition but lacking terminal inverted repeats.

This is only the second identification of a mutation caused by the insertion of an IS sequence in *S. typhimurium*. Casadesus and Roth (1) screened approximately 2,500 His^- mutants, and none appeared to have insertions. They suggest that transposable elements in *Salmonella* spp. may be able to distinguish between transcribed and nontranscribed regions or that target sites for insertion have been removed from essential genes. As the *gpt* gene is not essential for growth and is unlikely to be highly transcribed under normal growth conditions, the isolation of a *gpt*::IS*200* mutation does not allow us to discriminate between these two possibilities. Following the isolation of the *gpt-83* mutation, approximately 100 8-azaguanine-resistant mutants were screened by Southern blot analysis, but none showed insertions.

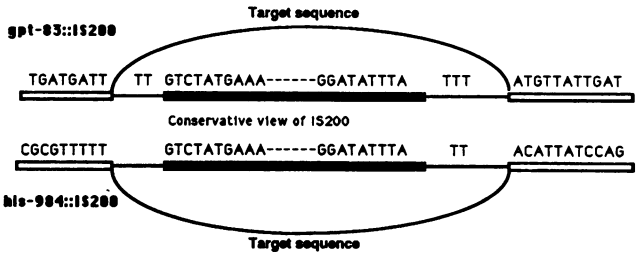

FIG. 4. Sequence comparison of *gpt-83*::IS*200* and *his-864*::IS*200*. Comparison of the IS*200* from the element found at the *hisD* locus (4) and the element found at the *gpt* locus. Open bars, Target sequences; solid bars, IS*200* sequence; thin lines, sequence that cannot be unambiguously assigned to either.

LITERATURE CITED

1. **Casadesus, J., and J. R. Roth.** 1989. Absence of mutations among spontaneous mutants of *Salmonella typhimurium*. Mol. Gen. Genet. **216**:210–216.
2. **Lam, S., and J. R. Roth.** 1983. IS*200*: a *Salmonella*-specific insertion sequence. Cell **34**:951–960.
3. **Lam, S., and J. R. Roth.** 1983. Genetic mapping of IS*200* copies in *Salmonella typhimurium* strain LT2. Genetics **105**:801–811.
4. **Lam, S., and J. R. Roth.** 1986. Structural and functional studies of insertion element IS*200*. J. Mol. Biol. **187**:157–167.
5. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Matsutani, S., H. Ohtsubo, Y. Maeda, and E. Ohtsubo.** 1987. Isolation and characterisation of IS elements repeated in the bacterial genome. J. Mol. Biol. **196**:445–455.
7. **O'Reilly, C., P. D. Turner, P. F. Smith-Keary, and D. J. McConnell.** 1984. Molecular cloning of genes involved in purine biosynthetic and salvage pathways of *Salmonella typhimurium*. Mol. Gen. Genet. **196**:152–157.
8. **Riley, M., C. O'Reilly, and D. J. McConnell.** 1983. Physical map of *Salmonella typhimurium* in the vicinity of the *proA* gene. J. Bacteriol. **157**:655–657.
9. **Sangar, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.