Dual effect of a Tn917 insertion into the *Bacillus subtilis* *sacX* gene

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The most common effect of transposon insertion is the inactivation of genes. However, in some cases, transposons can activate *in cis* the expression of genes in the neighbourhood of their integration site. We previously described an insertion of the transposon Tn917 into the *Bacillus subtilis* *sacXY* locus. *sacX* and *sacY* encode respectively a negative and a positive regulator involved in induction by sucrose of the exoenzyme levansucrase. Data in this paper show that the Tn917 insertion had two effects: it inactivated *sacX* and it increased the transcription of *sacY*. The latter effect involved one or several elements internal to the transposon.

Introduction

Transposons are mobile genetic elements whose insertion into genomes contributes to genetic variability (Berg & Howe, 1989). The most common effect of transposon insertion is the inactivation of the target gene. This can be due to interruption of the coding sequence, to genetic rearrangements (Reif & Saedler, 1975), or to negative polar effects when a transposon carrying a transcription terminator inserts between the promoter of a gene and its coding sequence (Galas & Chandler, 1989). Transposon insertions can also occasionally activate *in cis* the expression of silent or weakly expressed genes. The latter effect can be observed for example if an outward-directed promoter present within the transposon promotes transcription of external sequences (Ciampi *et al.*, 1982).

Youngman and coworkers have developed the *Streptococcus faecalis* transposon Tn917 as a tool for studying the genetics of *Bacillus subtilis* and other Gram-positive bacteria (Perkins & Youngman, 1984; Youngman *et al.*, 1985; Youngman, 1987). This transposon belongs to the Tn3 family. Its general organization, deduced from its nucleotide sequence (Shaw & Clewell, 1985), is presented in Fig. 1. We previously characterized a Tn917 insertion into the *B. subtilis* *sacXY* locus (formerly *sacS*) (Aymerich & Steinmetz, 1987), which is involved in the induction by sucrose of the *sacB*-encoded exoenzyme levansucrase (Lepesant *et al.*, 1976). This Tn917 insertion led to a constitutive levansucrase phenotype, which was therefore interpreted as being due to the inactivation of a gene (subsequently designated *sacX*) encoding a negative

regulator. Additional experiments showed that a second gene (designated *sacY*), downstream of *sacX* (Fig. 1), encoded a positive regulator of levansucrase induction

Fig. 1. Structure of the *sacXY* locus and of *sacX* mutations. The restriction map was deduced from the DNA sequence (Zukowski *et al.*, 1988, 1990). The insertion site of Tn917 into *sacX* was determined from the restriction map of plasmids pSL4 and pSL9 (Aymerich & Steinmetz, 1987). The *sacX*ΔI deletion (ΔI) is an in-frame deletion within *sacX* extending from the *ClaI* site to the *AvaI* site (Crutz *et al.*, 1990). Tn917, which is 5257 bp long (Shaw & Clewell, 1985), is not drawn to scale. It contains four open reading frames of more than fifty codons, oriented in the same direction (arrows). LE, left-hand end; RE, right-hand end; *erm*, erythromycin-resistance gene; *tnpR*, gene encoding resolvase; *orf5* and *orf6*, open reading frames probably encoding the transposase (Perkins & Youngman, 1984; Shaw & Clewell, 1985; Murphy, 1989).

(Aymerich & Steinmetz, 1987; Steinmetz *et al.*, 1988) and suggested that *SacX* was an inhibitor of *SacY* in the absence of sucrose (Crutz *et al.*, 1990). Since there is no obvious transcription terminator between *sacX* and *sacY* (Zukowski *et al.*, 1988, 1990), *sacY* could be transcribed, at least in part, from the *sacX* promoter. This prompted us to compare the phenotype of the *sacX::Tn917* mutant with that of mutants carrying other alterations of *sacX*.

We present here evidence that the *sacX::Tn917* insertion not only inactivated the gene for the *SacX* negative regulator, but also stimulated the expression of the gene coding for the *SacY* positive regulator. This observation shows that, as in the case of transposons of Gram-negative bacteria (Ciampi *et al.*, 1982), the phenotype of some *Tn917*-derived mutants may not simply be the result of insertional inactivation of the target gene. We also describe procedures that allow the other activities of inserted transposons to be recognized.

Methods

Bacterial strains. *Escherichia coli* strain TG1 (*supE hsdΔ5 thi Δ(lac-proAB) F[traD36 proAB⁺ lac^R lacZΔM15]*) was used as a host for the construction of plasmids. *Bacillus subtilis* strains are listed in Table 1. QB1072 and GM124 have been described previously (Aymerich & Steinmetz, 1987); GM275a is a GM124 derivative in which the *lacZ* gene present within the *Tn917* variant inserted into *sacX* was deleted in two steps as described in Fig. 2. GM276 and GM729 are derivatives of GM275a and QB1072, respectively, carrying the *sacB'-lacZ* fusion (Aymerich *et al.*, 1986). This fusion was introduced into the chromosome of these strains using plasmid pLG331 (Crutz *et al.*, 1990). GM687, GM688, GM689, GM690 and GM706 are derivatives of

GM276 transformed with pTV21Δ2, pIC42, pIC47, pIC48 and pIC41, respectively, as shown in Fig. 3. GM682 and GM730 are derivatives of QB1072 and GM729 respectively, in which the *sacXΔ1* deletion was introduced in two steps using pSL132 and pSL85-XΔ1 as previously described (Crutz *et al.*, 1990). GM684, GM685 and GM686 are derivatives of QB1072, GM275a and GM682, respectively, carrying the plasmid pSL170 inserted into *sacY* (Fig. 4).

Plasmids. The following plasmids have been described previously: pLG331, pSL85-XΔ1 and pSL132 (Crutz *et al.*, 1990); pTV8 and pTV21Δ2 (Youngman, 1987). Plasmid constructions and analyses were performed using standard techniques; ligations of incompatible ends were performed after filling extremities with DNA polymerase (Klenow fragment). Plasmid pIC11 (shown linearized at its *ClaI* site in Fig. 2) contains a 1825 bp *ClaI*-*PvuII* fragment of *lacZ*, the *PvuII*-*BglII* fragment of pTV21Δ2 (including the pBR322 origin and the chloramphenicol-resistance gene), and the right-hand end of *Tn917* minus the last 33 nucleotides (*BglII*-*ClaI* fragment). The construction of pTV21Δ2 derivatives pIC41 and pIC42 is shown in Fig. 3. Plasmids pIC47 and pIC48 were derived from pIC42 as described in the legend of Fig. 3. Plasmid pSL170 was derived from pJM783 (Perego *et al.*, 1988) and pSL150 (Crutz *et al.*, 1990) as described in the legend of Fig. 4.

Table 1. Bacterial strains

Strain	Genotype*	Origin†
QB1072	Wild-type	1
GM124	<i>sacX::Tn917-lac</i>	1
GM275	<i>sacX::Tn917-lac'::pIC11</i>	2
GM275a	<i>sacX::Tn917</i>	2
GM276	<i>sacX::Tn917, sacB'-lacZ</i>	2
GM682	<i>sacXΔ1</i>	2
GM684	<i>sacY::pSL170</i>	2
GM685	<i>sacX::Tn917, sacY::pSL170</i>	2
GM686	<i>sacXΔ1, sacY::pSL170</i>	2
GM687	<i>sacX::Tn917::pTV21Δ2, sacB'-lacZ</i>	2
GM688	<i>sacX::Tn917::pIC42, sacB'-lacZ</i>	2
GM689	<i>sacX::Tn917::pIC47, sacB'-lacZ</i>	2
GM690	<i>sacX::Tn917::pIC48, sacB'-lacZ</i>	2
GM706	<i>sacX::Tn917::pIC41, sacB'-lacZ</i>	2
GM729	<i>sacB'-lacZ</i>	2
GM730	<i>sacXΔ1, sacB'-lacZ</i>	2

* All the strains carry the *sacA321* mutation. The *Tn917* transposon carried by GM275a and derivatives is that carried by pTV8 (Youngman, 1987).

† 1, Aymerich & Steinmetz (1987); 2, this work.

Fig. 2. Construction of strain GM275a. (a) GM124 was transformed by pIC11 linearized by *ClaI*. Chloramphenicol-resistant (Cm^R) transformants (which were erythromycin sensitive) were selected. (b) The resulting strain GM275 was transformed by pTV8 linearized by *PstI*. Erythromycin-resistant (Em^R) transformants were selected. ori, pBR322 origin of replication; *cat*, chloramphenicol-resistance gene; *erm*, erythromycin-resistance gene; LE, left-hand end; RE, right-hand end; crosses, cross-over events. Truncated parts of genes or transposon are symbolized by primes (').

Fig. 3. Construction and insertion of plasmids into the chromosome of GM276. Plasmids pIC41 and pIC42 were derived from pTV21Δ2 by a *Sal*I/*Hpa*I (Δ*S*/*Hp*) and a *Xba*I/*Pvu*II (Δ*X*/*P*) deletion, respectively; the point corresponding to the deletion is indicated in pIC41 and pIC42 by a δ. Integration of these plasmids into the chromosome of GM276 led to GM706 and GM688, respectively. Plasmids pIC47 and pIC48 were derived from pIC42 by a *Sal*I/*Hind*III and a *Sal*I/*Bgl*II deletion, respectively; integration of these plasmids into the chromosome of GM276 led to GM689 and GM690, respectively, similar to GM688, except that they have respectively a *Sal*I/*Hind*III and a *Sal*I/*Bgl*III deletion (indicated by > Δ <) in the portion of the transposon remaining just upstream of *sacY*. The structure of GM687, in which part of the transposon is substituted by pTV21Δ2 sequences, is also shown. *B*, *H*, *Hp*, *P*, *S* and *X* represent *Bgl*II, *Hind*III, *Hpa*I, *Pvu*II, *Sal*I and *Xba*I restriction sites, respectively. The origin of replication of plasmids is represented by a black point. *cat*, chloramphenicol-resistance gene; *erm*, erythromycin-resistance gene; crosses, cross-over events; Tn' and Tn symbolize transposon Tn917 truncated of its right and left part, respectively.

Transformations, media and enzyme assays. *E. coli* and *B. subtilis* were transformed using standard protocols as described previously (Crutz *et al.*, 1990). Transformants were selected on LB solid medium (Miller, 1972) supplemented with appropriate antibiotics. When required, integration of a single plasmid copy into the *B. subtilis* chromosome was screened as previously described (Aymerich *et al.*, 1986). Growth of liquid cultures of *B. subtilis* in CG medium and preparation of enzyme extracts were done as previously described (Aymerich *et al.*, 1986). Assay of β-galactosidase was performed according to Miller (1972).

Results

Constitutivity in different *sacX* mutants

We looked at the effect of *sacX*::Tn917 on the expression of the downstream *sacY* gene by comparing the phenotype of two mutants, with isogenic back-

grounds, differently affected in *sacX*. These mutants carried the *sacB*'-'*lacZ* fusion, whose expression was previously shown to be regulated in an identical way to that of the *sacB* gene (Aymerich *et al.*, 1986). The *sacX*Δ*I* mutation is an in-frame deletion within the *sacX* coding sequence, in which about 46% of the codons are removed (Fig. 1). This mutation led to a constitutive expression of the *lacZ* reporter gene, as previously observed (Crutz *et al.*, 1990). Table 2 shows that the *sacX*::Tn917 mutant also expressed the *lacZ* reporter gene constitutively, but at a much higher level than that observed in the *sacX*Δ*I* mutant. Since SacY is an activator of transcription of *sacB*, the difference in phenotypes could reflect differences in the expression of *sacY*. In order to analyse a possible positive effect of the Tn917 sequences on *sacY* expression, we constructed strains in which the trans-

Fig. 4. Structure of plasmid pSL170 and insertion into *sacY*. The 613 bp *ScaI/SalI* fragment of pJM783 was replaced by a 723 bp *HincII/SalI* fragment from pSL150 carrying the 3' end of *sacX*, the 5' end of *sacY* and the *spoVG* ribosome-binding site (RBS, dotted area). This led to the integrative plasmid pSL170, which contains a *sacY'-lacZ* transcriptional fusion. A, C, H, S and Sc represent *AvaI*, *Clal*, *HincII*, *SalI* and *ScaI* restriction sites, respectively; (Sc/H) stands for the *ScaI/HincII* ligation, which destroyed both sites. *cat*, chloramphenicol-resistance gene; cross, cross-over event. Genes are represented by arrows, indicating their orientation. The positions of the Tn917 insertion and the *sacXΔ1* deletion are shown.

poson contained a variety of insertions. GM276 (*sacX::Tn917*) was transformed by plasmid pIC42, giving GM688, in which the region immediately upstream of *sacY* consists of a small fragment of Tn917 from its unique *SalI* site to the nearest transposon end; upstream of this *SalI* site lie plasmid sequences (Fig. 3). As shown in Table 2, the constitutive β -galactosidase activity was greatly reduced by this insertion. When GM276 was transformed by pTV21Δ2, integration of the plasmid occurred through a double recombination event (Youngman, 1987), giving GM687, in which part of the transposon upstream of *SalI* was replaced by plasmid sequences (Fig. 3). The level of β -galactosidase activity in this strain was comparable to that in GM688, i.e. greatly reduced (Table 2). Besides this, GM706, in which most of the transposon just upstream of *sacY* is intact, had a phenotype similar to that of GM276 (Table 2).

Table 2. Expression of the *sacB'-lacZ* fusion in GM729 and isogenic *sacX* mutants

Strain	Relevant genotype*	β -Galactosidase activity†
GM729	Wild-type	<0.1 (induced: 11.8)
GM730	<i>sacXΔ1</i>	6.9
GM276	<i>sacX::Tn917</i>	209.4
GM687	<i>sacX::Tn917::pTV21Δ2</i>	20.5
GM706	<i>sacX::Tn917::pIC41</i>	220.7
GM688	<i>sacX::Tn917::pIC42</i>	26.7
GM689	<i>sacX::Tn917::pIC47</i>	19.3
GM690	<i>sacX::Tn917::pIC48</i>	15.3

* All these strains carry the *sacB'-lacZ* fusion.

† Expressed in Miller units. These activities correspond to a typical experiment. Similar results were reproducibly obtained in several experiments.

Table 3. Effect of Tn917 on the expression of the *sacY'-lacZ* fusion

Strain	Relevant genotype	β -Galactosidase activity*
QB1072	Wild-type	<0.1
GM684	<i>sacY'-lacZ</i>	0.5
GM685	<i>sacY'-lacZ, sacX::Tn917</i>	3.6
GM686	<i>sacY'-lacZ, sacXΔ1</i>	0.5

* Expressed in Miller units. See footnote † of Table 2.

These results suggested that the region upstream of the *SalI* site on the transposon had a positive effect on *sacB* expression.

The *sacX::Tn917* insertion has a positive effect on *sacY* transcription

The phenotypes observed in different *sacX* mutants were likely to reflect the levels of the SacY positive regulator. In order to measure directly any activating effect of the *sacX::Tn917* insertion on *sacY* transcription, we constructed a transcriptional fusion of *sacY* and *lacZ* and introduced it into the chromosome of different *sacX* mutants (Fig. 4). The β -galactosidase activities of the transformants show that the *sacX::Tn917* insertion increased the expression of the *lacZ* reporter gene (Table 3), demonstrating the positive effect of the insertion on *sacY* transcription.

Effect of the Tn917 sequences located downstream of the *SalI* site

The level of β -galactosidase produced constitutively by strain GM688 (*sacX::Tn917::pIC42*) was significantly higher than that of GM730 (*sacXΔ1*). This difference could have been due to a weak residual positive effect of

the part of Tn917 remaining just upstream of *sacY* in GM688. Therefore, we created deletions in this region by constructing strains GM689 and GM690, in which the remaining part of Tn917 was shortened from the *SalI* site (Fig. 3). The level of β -galactosidase activity in these strains was reduced as compared to that of GM688, and the reduction correlated with the extent of the deletion (Table 2). This result showed that the Tn917 region downstream of *SalI* had a minor, positive effect on expression of *sacY* in the *sacX::Tn917* mutant.

Discussion

Using a *sacY-lacZ* transcriptional fusion, we showed that the *sacX::Tn917* insertion stimulated transcription of the *sacY* gene located downstream of *sacX* (Table 3). The increased transcription of *sacY* led to a constitutive expression of *sacB* that was much stronger than that observed in a *sacX Δ I* mutant or in an induced wild-type strain (Table 2). This supports the suggestion that SacY is limiting for the induction of *sacB* in the wild-type strain (Henner *et al.*, 1987; Crutz *et al.*, 1990).

The question remains as to what is the basis of the effect of Tn917. Relatively little is known about the transcriptional activity of Tn917 and its effects on the transcription of genes adjacent to the integration site. Shaw & Clewell (1985) have shown that, in a *S. faecalis* strain carrying the transposon on a plasmid, several Tn917-specific transcripts are present. Some of these transcripts start from the *erm* promoter and extend towards the right-hand end of the transposon. The influence of insertions in GM687 (*sacX::Tn917::pTV21 Δ 2*) and GM688 (*sacX::Tn917::pIC42*) on β -galactosidase activity could be due to the presence of the chloramphenicol-resistance gene (*cat*) and its terminator between the *erm* promoter and *sacY*. As previously shown by Vandeyar *et al.* (1986), a *cat* insertion into Tn917 had a strong polar effect on the expression of downstream genes in *B. subtilis*.

Still, there was a residual constitutive β -galactosidase activity in GM687 and GM688, which could have been due to leakage through the *cat* terminator, or to other elements, e.g. a promoter, in the part of the transposon located downstream of the *SalI* site. Deletions in this region led to reduced levels of β -galactosidase activity, and the effect of these deletions was correlated with their extent, contrary to what might be expected for the deletion of a promoter. Alternatively, the effect of this region could be to stabilize messenger RNA transcripts initiated upstream and extending through *sacY* (Tanaka & Kawata, 1986), or to enhance the activity of a promoter for *sacY* located at the very end of Tn917 or

even downstream, in *B. subtilis* sequences (Reynolds *et al.*, 1981).

Although our experiments did not allow us to localize precisely an activating sequence, nor to be certain about the involvement of one or several promoter(s), it is clear that several elements internal to the transposon are involved in the stimulation of *sacY* transcription. This observation illustrates a limitation of transpositional mutagenesis as a tool for investigating the control of gene expression. The work presented here suggests a two-step strategy in order to distinguish between the effects of transposon insertions. In the first place, the phenotype conferred by a Tn917::pTV21 Δ 2 insertion should be different from that of the transposon alone. This difference could be due either to a phenomenon analogous to the one presented in this paper or to a polar effect of the *cat* gene (Vandeyar *et al.*, 1986). Secondly, the use of plasmids of the pIC41 type, which lead to the insertion of a *cat* gene without affecting the promoter activity of the transposon (Fig. 3), allows these two possibilities to be discriminated easily.

References

- AYMERICH, S. & STEINMETZ, M. (1987). Cloning and preliminary characterization of the *sacS* locus from *Bacillus subtilis* which controls the regulation of the exoenzyme levansucrase. *Molecular and General Genetics* **208**, 114–120.
- AYMERICH, S., GONZY-TREBOUL, G. & STEINMETZ, M. (1986). 5'-Noncoding region *sacR* is the target of all identified regulation affecting the levansucrase gene in *Bacillus subtilis*. *Journal of Bacteriology* **166**, 993–998.
- BERG, D. E. & HOWE, M. M. (editors) (1989). *Mobile DNA*. Washington, DC: American Society for Microbiology.
- CIAMPI, M. S., SCHMID, M. B. & ROTH, J. R. (1982). Transposon Tn10 provides a promoter for transcription of adjacent genes. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 5016–5020.
- CRUTZ, A. M., STEINMETZ, M., AYMERICH, S., RICHTER, R. & LE COQ, D. (1990). Induction of levansucrase in *Bacillus subtilis*: an antitermination mechanism negatively controlled by the phosphotransferase system. *Journal of Bacteriology* **172**, 1043–1050.
- GALAS, D. J. & CHANDLER, M. (1989). Bacterial insertion sequences. In *Mobile DNA*, pp. 109–162. Edited by D. E. Berg & M. M. Howe. Washington DC: American Society for Microbiology.
- HENNER, D. J., YANG, M., BAND, L., SHIMOTSU, H., RUPPEN, M. & FERRARI, E. (1987). Genes of *Bacillus subtilis* that regulate the expression of degradative enzymes. In *Genetics of Industrial Microorganisms (Proceedings of the Fifth International Symposium on the Genetics of Industrial Microorganisms)*, pp. 81–90. Edited by M. Alacevic, D. Hranueli & Z. Toman. Karlovac: Ognjen Prica Printing Works.
- LEPESANT, J. A., KUNST, F., PASCAL, M., LEPESANT-KEJZLAROVA, J., STEINMETZ, M. & DEDONDER, R. (1976). Specific and pleiotropic regulatory mechanisms in the sucrose system of *B. subtilis* 168. In *Microbiology 1976*, pp. 58–69. Edited by D. Schlessinger. Washington DC: American Society for Microbiology.
- MILLER, J. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MURPHY, E. (1989). Transposable elements in Gram-positive bacteria. In *Mobile DNA*, pp. 269–288. Edited by D. E. Berg & M. M. Howe. Washington DC: American Society for Microbiology.

- PEREGO, M., SPIEGELMAN, G. B. & HOCH, J. A. (1988). Structure of the gene for the transition state regulator, *abrB*: regulator synthesis is controlled by the *spoOA* sporulating gene in *Bacillus subtilis*. *Molecular Microbiology* **2**, 689–699.
- PERKINS, J. B. & YOUNGMAN, P. J. (1984). A physical and functional analysis of Tn917, a *Streptococcus* transposon in the Tn3 family that functions in *Bacillus*. *Plasmid* **12**, 119–138.
- REIF, H. J. & SAEDLER, H. (1975). IS1 is involved in deletion formation in the *gal* region of *Escherichia coli* K-12. *Molecular and General Genetics* **137**, 17–18.
- REYNOLDS, A. E., FELTON, J. & WRIGHT, A. (1981). Insertion of DNA activates the cryptic *bgl* operon in *E. coli* K-12. *Nature, London* **293**, 625–629.
- SHAW, J. H. & CLEWELL, D. B. (1985). Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon Tn917 in *Streptococcus faecalis*. *Journal of Bacteriology* **164**, 782–796.
- STEINMETZ, M., AYMERICH, S., LE COQ, D. & GONZY-TREBOUL, G. (1988). Levansucrase induction by sucrose in *Bacillus subtilis* involves an antiterminator. Homology with the *Escherichia coli bgl* operon. In *Genetics and Biotechnology of Bacilli*, vol 2, pp. 11–16. Edited by A. T. Ganesan & J. A. Hoch. New York & London: Academic Press.
- TANAKA, T. & KAWATA, M. (1986). Enhanced gene expression in *Bacillus subtilis* by *Escherichia coli* insertion sequences, IS5 and IS1. In *Bacillus Molecular Genetics and Biotechnology Applications*, pp. 467–477. Edited by A. T. Ganesan & J. A. Hoch. New York & London: Academic Press.
- VANDEYAR, M. A., MACKEY, C. J., LIPSKY, R. H. & ZAHLER, S. A. (1986). The *ilvBC-leu* operon of *Bacillus subtilis*. In *Bacillus Molecular Genetics and Biotechnology Applications*, pp. 295–305. Edited by A. T. Ganesan & J. A. Hoch. New York & London: Academic Press.
- YOUNGMAN, P. (1987). Plasmid vectors for recovering and exploiting Tn917 transpositions in *Bacillus* and other Gram-positive bacteria. In *Plasmids: a Practical Approach*, pp. 79–103. Edited by K. Hardy. Oxford: IRL Press.
- YOUNGMAN, P., ZUBER, P., PERKINS, J. B., SANDMAN, K., IGO, M. & LOSICK, R. (1985). New ways to study developmental genes in spore-forming bacteria. *Science* **228**, 285–290.
- ZUKOWSKI, M., MILLER, L., COGSWELL, P. & CHEN, K. (1988). An inducible expression system based on sucrose metabolism genes of *Bacillus subtilis*. In *Genetics and Biotechnology of Bacilli*, vol. 2, pp. 17–22. Edited by A. T. Ganesan & J. A. Hoch. New York & London: Academic Press.
- ZUKOWSKI, M., MILLER, L., COGSWELL, P. & CHEN, K., AYMERICH, S. & STEINMETZ, J. (1990). Nucleotide sequence of the *sacS* locus of *Bacillus subtilis* reveals the presence of two regulatory genes. *Gene* **90**, 153–155.