Transformation of *Arthrobacter* and studies on the transcription of the *Arthrobacter ermA* gene in *Streptomyces lividans* and *Escherichia coli*

Anne N. ROBERTS,* Leslie BARNETT and Sydney BRENNER
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

We report the development of a plasmid-mediated transformation system for *Arthrobacter* sp. NRRLB3381, using the *Streptomyces* cloning vector pIJ702. Our procedure gives a transformation frequency of $10^3/\mu\text{g}$ of plasmid DNA. In addition we have explored the expression of the *Arthrobacter ermA* gene in *Streptomyces lividans* and *Escherichia coli*, and shown that the *ermA* promoter is recognized in *S. lividans* not *E. coli*. The relationship between *Arthrobacter*, *Streptomyces* and *E. coli* promoters is discussed.

INTRODUCTION

Arthrobacter belongs to the Coryneform group of bacteria, which also includes such genera as *Corynebacterium*, *Cellulomonas*, *Brevibacterium*, *Curtobacterium* and *Microbacterium*. Interest in the genus *Arthrobacter* stems from the fact that its members have an extensive secondary metabolism. Relevant features that have been assigned to at least some *Arthrobacter* species include the capacity to degrade herbicides, pesticides and chitin, steroid transformation, and the ability to produce antibiotics, phytohormones, polysaccharides and riboflavin. In general the group is nutritionally versatile and can use a diverse range of organic substrates (Keddie & Jones, 1981). The particular species of *Arthrobacter* we have studied (NRRLB3381) possesses a cell wall containing L-diaminopimelic acid and a genome composed of 70.5% G+C, two properties that distinguish the *simplex* group of arthrobacters (DNA G+C content of 70–74%; Keddie & Jones, 1981) but which are shared by the *Streptomyces*. In addition, *Arthrobacter* sp. NRRLB3381, like *Streptomyces erythreus*, is able to produce the macrolide antibiotic erythromycin (French *et al.*, 1970). However, *Arthrobacter* sp. NRRLB3381 differs from *Streptomyces* in two significant ways: it is asporogenous and it grows as unicellular isolated colonies, not as a mycelium characteristic of *Streptomyces*. These features may facilitate the use of this organism for the expression of cloned genes, and may offer certain advantages over *Streptomyces*. We have developed plasmid-mediated transformation of *Arthrobacter* sp. NRRLB3381 to investigate the utility of *Streptomyces* cloning vectors in *Arthrobacter*. The expression of the *Arthrobacter ermA* gene in *S. lividans* and *E. coli* is also analysed.

MATERIALS AND METHODS

Bacterial strains

Arthrobacter sp. NRRLB3381 (French *et al.*, 1970) and *Streptomyces lividans* 1326 (Schottel *et al.*, 1981) were used for this study. The culture conditions for these two organisms have been described (Roberts *et al.*, 1985;

Chater *et al.*, 1982). *Arthrobacter* sp. NRRLB3381 will be simply referred to as *Arthrobacter* throughout the rest of the paper.

Transformation

S. lividans was transformed by standard methods (Chater *et al.*, 1982). A transformation procedure for *Arthrobacter* was adapted from the *Streptomyces* protocol (Hopwood *et al.*, 1985). Buffer P, R2YE plates and soft nutrient agar were made as described (Chater *et al.*, 1982; Hopwood *et al.*, 1985). An overnight culture of *Arthrobacter* was diluted 1:10 and grown for 4 h at 30 °C. The following procedure applies to a 10 ml culture sample. The cells were harvested by centrifugation at 5000 rev./min and 22 °C for 7.5 min. The pellet was washed with 7.5 ml of 10.3% sucrose and resuspended in 2 ml of P buffer containing 5 mg of lysozyme/ml. The culture was incubated at 30 °C with intermittent shaking. After 1 h, 2.5 ml of P buffer was added and incubation continued for a further 1 h, resulting in the formation of osmotically sensitive protoplasts. These were centrifuged at 5000 rev./min and 22 °C for 10 min, washed with 5 ml of P buffer and then resuspended in 0.3 ml of P buffer. Aliquots (50 μl) of this protoplast suspension, dispensed in Minifuge tubes, were used for each transformation. DNA, contained in a volume of 1–10 μl , was added and mixed by gently tapping the tube. This was followed immediately by the addition of 200 μl of P buffer containing 50% poly(ethylene glycol) 1000 (Koch-Light) and pipetting slowly up and down four times in order to mix. The sample was diluted with 750 μl of P buffer and the tube was inverted two or three times. Appropriate dilutions of this mixture were plated out directly on R2YE agar and incubated at 30 °C for approx. 24 h, or until faint growth was just visible. Thiostrepton-resistant transformants were selected by overlaying the plates with 2.5 ml of soft nutrient agar containing 600 μg of the antibiotic/ml. Transformants were scored after 5–6 days at 30 °C.

Preparation of DNA and cloning techniques

General methods used for cloning steps, preparation of DNA and nick-translation have been described

* Present address and address for reprint requests: Department of Biological Sciences, Stanford University, Stanford, CA 94305–2493, U.S.A.

Table 1. 'Prime, cut' probes used for S1 mapping and primer extension experiments

Each probe is named after the number of the M13 clone and the restriction enzyme used for cutting. The size of each probe includes 48 nucleotides of M13 sequence (51 nucleotides for *EcoRI*-cut probes). Each probe is complementary to the non-coding sequence of the *ermA* gene described in Roberts *et al.* (1985).

Name	Positions covered in <i>ermA</i> sequence	Size (nucleotides)
23.X/ <i>SmaI</i>	303-4	299*
03.X/ <i>BglII</i>	443-155	336
03.X/ <i>ApaI</i>	443-294	197
10.X/ <i>BglII</i>	485-155	378
41.X/ <i>ApaI</i>	659-294	413
22.X/ <i>NcoI</i>	695-336	407
06.S/ <i>EcoRI</i>	1410-1191	270
64.S/ <i>NciI</i>	1457-1249	256
61.S/ <i>EcoRI</i>	1644-1429	266
56.S/ <i>PvuII</i>	1822-1556	314

* Clone 23.X contains an additional *SmaI* site which was formed fortuitously when insert DNA was blunt-end ligated into the *SmaI* site of mp8. Therefore the size of the probe does not include any M13 sequence since cleavage with *SmaI* totally removes all M13 DNA.

(Roberts *et al.*, 1985). Plasmid DNA from *S. lividans* was prepared by the method of Hopwood *et al.* (1983). Plasmid DNA was extracted from *Arthrobacter* cells by the Birnboim & Doly (1979) method, after incubation in lysozyme (5 mg/ml) at 30 °C for 1 h. Genomic DNA was prepared as described by Chater *et al.* (1982)

Subcloning *ermA* into *Streptomyces lividans*

A 4 kb *BamHI* fragment containing the entire *ermA* gene (previously cloned into pUC8; Roberts *et al.*, 1985) was subcloned into the *BglII* site of the multicopy plasmid pIJ702 (Katz *et al.*, 1983) and transformed into *S. lividans* 1326 (Schottel *et al.*, 1981). This construct was called pAR4.

Transcript analysis

Transcription of the *ermA* gene, cloned in pIJ702 (pAR4) in *S. lividans* or cloned in pUC8 (pAR2) in *E. coli*, was analysed by S1 mapping and primer extension studies. These experiments were carried out using 'prime, cut' probes prepared on M13 templates that hybridized to the appropriate regions of RNA. The method by which RNA was prepared, conditions for S1 mapping and primer extension analysis and formation of 'prime, cut' probes were described previously (Roberts *et al.*, 1985). Table 1 lists the probes prepared.

RESULTS AND DISCUSSION

Plasmid-mediated transformation of *Arthrobacter*

The method used to transform *Arthrobacter*, adapted from the standard *Streptomyces* protocol (Hopwood *et al.*, 1985), is described in the Materials and methods section. Many changes to this procedure simply reflect

physical differences between the unicellular *Arthrobacter* and mycelial *Streptomyces*. Additional significant changes were: (i) a higher lysozyme concentration which improved the yield of osmotically sensitive protoplasts, (ii) an increased incubation time in lysozyme, although as for *Streptomyces* this was not critical; protoplast formation was monitored by phase-contrast microscopy and cells were routinely used after 1.5-2 h, and (iii) in our hands a higher poly(ethylene glycol) concentration improved *Streptomyces* transformation frequency and was therefore adopted for *Arthrobacter*. Using these conditions we could demonstrate that 5-10% of the cells regenerated and of those that regenerated 99% were osmotically fragile. The *Streptomyces* plasmid pIJ702 which confers thiostrepton resistance was used for all transformations (Hopwood *et al.*, 1985). Confirmation that pIJ702 was transforming *Arthrobacter* to thiostrepton resistance was obtained from Southern analysis of total DNA prepared from three putative transformants. These DNA samples, cut with *SmaI* and *BamHI* gave the identical banding pattern as pure pIJ702 when probed with nick-translated pIJ702 (results not shown). Examination of plasmid DNA prepared from seven thiostrepton-resistant transformants revealed the presence of a vector the same size (5.8 kb) as pIJ702. This DNA also hybridized with nick-translated pIJ702. The plasmid DNA prepared from *Arthrobacter* transformed *S. lividans* to thiostrepton resistance and melanin secretion characteristic of pIJ702 (Katz *et al.*, 1983). From these experiments it was concluded that pIJ702 can transform *Arthrobacter* to thiostrepton resistance and can replicate autonomously. The plasmid is maintained in its entirety since its total size, the position of restriction sites and ability to transform *S. lividans* are preserved. In the absence of thiostrepton, the plasmid is maintained stably for at least seven generations as judged by the plate cell count in the presence and absence of antibiotic. We obtained a frequency of 10^3 transformants/ μ g of pIJ702 plasmid DNA.

Expression of erythromycin resistance from *ermA* in *Streptomyces lividans*

We have previously described (Roberts *et al.*, 1985) the cloning and characterization of a gene (*ermA*) coding for a presumed erythromycin resistance determinant isolated from an erythromycin-producing strain of *Arthrobacter* sp. NRRLB3381. The identity of the gene was established by (i) cross-hybridization to the *S. erythraeus* erythromycin-resistance gene *ermE*, and (ii) the predicted amino-acid sequence of the protein encoded by *ermA* showed substantial homology to three other erythromycin resistance determinants.

The plasmid pAR4 (described in the Materials and methods section) which contains the *ermA* gene was transformed into *S. lividans* by selecting for thiostrepton resistance. It was found that the thiostrepton-resistant transformants obtained had also acquired erythromycin resistance. All of these colonies were white in colour since cloning into the *BglII* site of pIJ702 inactivates the melanin-producing tyrosinase (*mel*) gene. Restriction analysis (results not shown) confirmed that the 4 kb *BamHI* fragment had been correctly inserted into pIJ702, producing an erythromycin-resistant derivative of this vector. In each of ten erythromycin-resistant transformants examined, the *ermA* gene was inserted in the same orientation as the *mel* promoter of pIJ702 (Hopwood

et al., 1985). This confirms the previous assignment of erythromycin resistance to the *ermA* gene.

Transcription from the *Arthrobacter ermA* promoter in *Streptomyces lividans*

It has been demonstrated that *Streptomyces*, like *E. coli* (Grossman *et al.*, 1984; Landick *et al.*, 1984), *Bacillus* (Losick & Pero, 1981) and recently the nitrogen-regulated genes of enteric bacteria (Hirschman *et al.*, 1985) possess multiple sigma (σ) factors (Westpheling *et al.*, 1985; Buttner & Brown, 1985) which are each believed to initiate transcription from a specific class of promoter. In *S. coelicolor* one of the σ -factors identified, probably the σ^{40} found by Buttner & Brown (1985) or σ^{35} of Westpheling *et al.* (1985), may be analogous to the *E. coli* σ^{70} factor that recognizes the consensus promoter of TTGACA (−35 region) and TATAAT (−10 region), and may be partly responsible for the capacity of *Streptomyces* to recognize and initiate transcription from promoters derived from a diverse source of bacteria including *E. coli* (Bibb & Cohen, 1982; Jaurin & Cohen, 1984; Westpheling *et al.*, 1985; Herbert *et al.*, 1986).

As an increasing number of *Streptomyces* genes have been sequenced and S1 mapped and promoter probe vectors become available (Brawner *et al.*, 1985; Ward *et al.*, 1986), it appears that most *Streptomyces* promoters belong loosely into one of two groups. The first group shows no similarity with the *E. coli* and vegetative *Bacillus* consensus promoter and includes such promoters as that from the hygromycin B phosphotransferase (HPH) gene from *S. hygrosopicus* (Zalacain *et al.*, 1986), the rRNA methylase (*tsr*) gene from *S. azureus*, the aminoglycoside phosphotransferase (*aph*) gene from *S. fradiae*, the viomycin phosphotransferase (*vph*) gene from *S. vinaceus* (Bibb *et al.*, 1985b; Janssen *et al.*, 1985) and the endoglycosidase-H (*endoH*) gene from *S. plicatus* (Westpheling *et al.*, 1985). Not surprisingly, those promoters tested for transcriptional activity in *E. coli* (*tsr*, *aph* and *vph*) do not function (Bibb *et al.*, 1985b).

Shown in Table 2 is the second group of *Streptomyces* promoter which is characterized by at least a partial similarity to the *E. coli* canonical promoter sequence. Comparison of these sequences enables a consensus sequence of TtGac_a^g (−35 region) and tAgaaT (−10

region) to be reached, in which capital letters indicate the bases most strongly conserved. It has been proposed that these promoters may be analogous to those read by σ^{70} of *E. coli* or σ^{55} of *Bacillus* and may represent the *Streptomyces* 'vegetative' promoter (Bibb *et al.*, 1985a). It would be necessary therefore, for the *Streptomyces* RNA polymerase to have less stringent promoter sequence requirements than *E. coli* RNA polymerase. Alternatively, *Streptomyces* may possess many more σ -factors than other bacteria, each of which recognizes a specific subset of promoter. Of those sequences tested for their promoter activity in *E. coli*, SEP2, SEP3 and SEP6, which closely match the *E. coli* promoter sequence, are active and the promoter pIJ101A, which differs considerably from the *E. coli* sequence, functions only weakly.

The *Arthrobacter ermA* promoter (Table 2) shows partial similarity to the *E. coli* consensus promoter. The transcription initiation site of the *ermA* gene in *S. lividans* was determined by S1 mapping using probe 10.X/BgII (see Table 1) which extends over the transcription start site of this gene in *Arthrobacter* and which previously protected a fragment of 200 nucleotides (Roberts *et al.*, 1985). The lower arrow in Fig. 1a shows that RNA from *Arthrobacter* (lane 3) and from *S. lividans* containing plasmid pAR4 (lane 4) protected the same sized fragment (197 nucleotides) from S1 nuclease digestion, strongly suggesting that transcription of *ermA* in *S. lividans* begins from the *Arthrobacter* promoter. This was confirmed by a primer extension experiment (Fig. 1b) which showed that transcription of *ermA* in *S. lividans* containing pAR4 (lane 3) proceeds from the same two residues as found in *Arthrobacter* (lanes 2, 4 and 5). Although there is a slight 'smile' in the gel, it is clear, nevertheless, that the *S. lividans* primer extension corresponds to the same position as that of *Arthrobacter*. From the equivalent gel of primer extension of the *ermA* gene in *Arthrobacter* (Roberts *et al.*, 1985), the transcription start of the *ermA* gene was assigned to an A at 286 and a G at 289, complementary to the T and C residues shown in Fig. 1b. The upper arrow in Fig. 1a denotes a band corresponding to full length protection of the probe by pAR4 RNA (lane 4) which probably corresponds to transcriptional read-through from the *mel* promoter of pIJ702 (Hopwood *et al.*, 1985). These

Table 2. *Streptomyces* promoter sequences

Promoter	−35 sequence	Space (bp)	−10 sequence	Reference
<i>E. coli</i> and vegetative	TTGACA	17±1	TATAAT	Moran <i>et al.</i> (1982)
<i>Bacillus</i>				Rosenberg & Court (1979)
<i>ermE</i> P1	TGGACA	14	TAGGAT	Bibb <i>et al.</i> (1985a)
<i>ermE</i> P2	TTGACG	18	GAGGAT	Bibb <i>et al.</i> (1985a)
ORF	CTGCGA	18	TAGCAT	Bibb <i>et al.</i> (1985a)
<i>gyl</i> P1	TTGACG	17	GAGACT	Janssen <i>et al.</i> (1985)
<i>gyl</i> P2	TCGAAC	19	TAGAGT	Janssen <i>et al.</i> (1985)
<i>gylR</i>	TTGGCG	19	TAGGTT	Janssen <i>et al.</i> (1985)
SEP2	TTGACG	18	TAAAAT	Jaurin & Cohen (1985)
SEP3	TTGACA	16	CATCAT	Jaurin & Cohen (1985)
SEP6	TGGACA	17	TTATAT	Jaurin & Cohen (1985)
pIJ101A	TTGCGC	18	CAGACT	Buttner & Brown (1985)
<i>Streptomyces</i> 'consensus'	TtGac _a ^g	18	tAgaaT	
<i>Arthrobacter ermA</i>	TCGGAC	16	TATCCT	Roberts <i>et al.</i> (1985)

Fig. 1. (a) S1 mapping of RNA from *Arthrobacter* and *S. lividans* containing pAR4 using 'prime, cut' probe 10.X/*Bgl*I described in Table 1 and (b) primer extension analysis using 'prime, cut' probe 03.X/*Apa*I

(a) Lane 1, probe without S1 nuclease treatment; lane 2, probe alone; lane 3, probe with *Arthrobacter* RNA; lane 4, probe with RNA from *S. lividans* containing pAR4. The marker positions in nucleotides refer to an end-labelled *Msp*I digest of pAT153. The arrows indicate the bands described in the Results and discussion section. (b) Lane 1, probe extended in the absence of RNA; lane 2, probe extended when annealed to *Arthrobacter* RNA; lane 3, probe extended when annealed to RNA from *S. lividans* containing pAR4; lane 4, probe extended when annealed to *Arthrobacter* RNA prepared by the method of Jaurin & Cohen (1984); lane 5, probe extended when annealed to *Arthrobacter* RNA prepared from a culture grown in the presence of 20 µg of exogenous erythromycin/ml. On the left hand side (lanes T, C, G and A) are shown the dideoxynucleotide sequencing reactions for clone 03.X. The two bases corresponding to the 3' end of each cDNA (T and C) are indicated by arrows.

results clearly demonstrate that the *S. lividans* RNA polymerase recognizes the *Arthrobacter* *ermA* promoter and initiates transcription at precisely the same position as the RNA polymerase of *Arthrobacter*. We find no evidence for transcription of the *ermA* gene proceeding from two start sites, as found for the *ermE* gene in *S. erythraeus* (Bibb *et al.*, 1985a).

The available evidence indicates that there are few heterospecific barriers affecting transcription between *Arthrobacter* and *Streptomyces*: the *Streptomyces* vector pIJ702 replicates autonomously and the *S. azureus* *tsr* gene, which contains a promoter that differs from the *E. coli* consensus, is expressed in *Arthrobacter*; the *Arthrobacter* *ermA* gene is expressed in *S. lividans*.

Since *tsr* and *ermA* represent the two groups of *Streptomyces* promoter so far discussed, it is likely that other *Streptomyces* genes are expressed in *Arthrobacter*. Thus it may be possible to take advantage of detailed molecular biology that has been carried out in *Streptomyces* for the development of cloning vectors in *Arthrobacter*.

Transcription of the *ermA* gene in *Escherichia coli*

The effectiveness of the *ermA* promoter in *E. coli* was assessed by galactokinase assays and S1 mapping. A fragment of DNA (extending from bp 158 to 307 of the sequenced *ermA* gene; Roberts *et al.*, 1985) containing the *ermA* promoter was inserted in the appropriate

orientation into the promoter-probe vector pKO500 (McKenny *et al.*, 1981). No galactokinase activity was detected (results not shown), indicating that the *ermA* promoter does not function in *E. coli*. This was confirmed by S1 mapping RNA derived from *E. coli* containing pAR2. This vector comprises the entire *ermA* gene contained on a 4 kb *Bam*HI fragment cloned into the *Bam*HI site of pUC8 (Roberts *et al.*, 1985). Restriction mapping characterized two isolates: pAR2A in which the *ermA* gene opposes the *lacZ* promoter of pUC8, and pAR2B in which the *ermA* gene is in the same orientation as *lacZ*. S1 mapping was carried out using 03.X/*Bgl*II (results not shown) which extends over the transcription start point of the *ermA* gene in *Arthrobacter*. RNA from pAR2A did not protect the probe from S1 nuclease activity, whereas RNA from pAR2B fully protected probe 03.X/*Bgl*II and all other probes tested (23.X/*Sma*I, 41.X/*Apa*I, 22.X/*Nco*I, 06.S/*Eco*RI, 64.S/*Nci*II, 61.S/*Eco*RI and 56.S/*Pvu*II; see Table 1) from S1 nuclease digestion (results not shown). These probes mapped regions of RNA between bp 4 and 1822 of the sequenced *ermA* gene. These results indicate that transcription of the *ermA* gene does not proceed from its own promoter in *E. coli* in either pAR2A or pAR2B. In pAR2B, transcription of *ermA* is initiated further upstream, presumably from the *lacZ* promoter of the vector. Taken together, these results demonstrate that, although the *ermA* promoter is partially homologous to the *E. coli* consensus promoter and has a near optimal spacing of 16 bp between the -10 and -35 region, it is not sufficiently similar to meet the relatively more stringent requirements of the *E. coli* RNA polymerase.

Relationship between the expression of erythromycin resistance genes

Erythromycin resistance genes examined are either expressed constitutively or induced by erythromycin by a translational attenuation mechanism (Dubnau, 1984). Genes that are inducible by erythromycin are characterized by a leader sequence preceding the coding sequence (Gryczan *et al.*, 1980, 1984; Horinouchi & Weisblum, 1980; Horinouchi *et al.*, 1983; Murphy, 1985) which is absent from constitutively expressed genes (Lampson & Parisi, 1986; Monod *et al.*, 1986). Examination of the nucleotide sequence and S1 mapping data of the erythromycin resistance genes *ermA* and *ermE* from two organisms that produce the antibiotic, *Arthrobacter* and *S. erythraeus* (Roberts *et al.*, 1985; Bibb *et al.*, 1985a; Uchiyama & Weisblum, 1985), show that a leader sequence is not present. Therefore it seems reasonable to propose that in organisms producing erythromycin, e.g. *Arthrobacter* and *S. erythraeus*, the gene responsible for erythromycin resistance is constitutively expressed. If antibiotic resistance genes originated in antibiotic-producing organisms (Benveniste & Davies, 1973; Walker & Walker, 1970) then it would appear that the inducibility of erythromycin resistance in non-antibiotic-producing bacteria must have been acquired later.

We thank Mr. S. J. Lucania of E. J. Squibb and Sons, New Brunswick, NJ, U.S.A. for the gift of thiostrepton, and Graham Hudson, Christine McKillop, Eric Miller, Andrew Travers, John Todd and Mary Waye for their encouragement and support. We also appreciate the advice of David Hopwood and his colleagues throughout the course of this study.

REFERENCES

- Benveniste, R. & Davies, J. (1973) Proc. Natl. Acad. Sci. U.S.A. **70**, 2276-2280
- Bibb, M. J. & Cohen, S. N. (1982) Mol. Gen. Genet. **187**, 265-277
- Bibb, M. J., Janssen, G. R. & Ward, J. M. (1985a) Gene **38**, 215-226
- Bibb, M. J., Bibb, M. J., Ward, J. M. & Cohen, S. N. (1985b) Mol. Gen. Genet. **199**, 26-36
- Birnboim, H. C. & Doly, J. (1979) Nucleic Acids Res. **7**, 1513-1523
- Browner, M. E., Auerbach, J. I., Fornwald, J. A., Rosenberg, M. & Taylor, D. P. (1985) Gene **40**, 191-201
- Buttner, M. J. & Brown, N. L. (1985) J. Mol. Biol. **185**, 177-188
- Chater, K. F., Hopwood, D. A., Kieser, T. & Thompson, C. J. (1982) Curr. Top. Microbiol. Immunol. **96**, 69-95
- Dubnau, D. (1984) CRC Crit. Rev. Biochem. **16**, 103-132
- French, J. C., Howells, J. D. & Anderson, L. E. (1970) U.S. Patent 3.551.294
- Grossman, A. D., Erickson, J. W. & Gross, C. A. (1984) Cell **38**, 383-390
- Gryczan, T. J., Grandi, G., Hahn, J., Grandi, R. & Dubnau, D. (1980) Nucleic Acids Res. **8**, 6081-6097
- Gryczan, T., Israeli-Reches, M., Del Bue, M. & Dubnau, D. (1984) Mol. Gen. Genet. **194**, 349-356
- Herbert, C. J., Sarwar, M., Ner, S. S., Giles, I. G. & Akhtar, M. (1986) Biochem. J. **233**, 383-393
- Hirschman, J., Wong, P.-K., Sei, K., Keener, J. & Kustu, S. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 7525-7529
- Hopwood, D. A., Kieser, T., Wright, H. M. & Bibb, M. J. (1983) J. Gen. Microbiol. **129**, 2257-2269
- Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P., Ward, J. M. & Schrempf, H. (1985) Genetic Manipulation of Streptomyces: A Laboratory Manual, The John Innes Foundation, Norwich
- Horinouchi, S. & Weisblum, B. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 7079-7083
- Horinouchi, S., Byeon, W.-H. & Weisblum, B. (1983) J. Bacteriol. **154**, 1252-1262
- Janssen, G. R., Bibb, M. J., Smith, C. P., Ward, J. M., Kieser, T. & Bibb, M. J. (1985) in Microbiology 1985 (Hegemon, G. & Hershberger, C. L., eds.), pp. 392-396, American Society for Microbiology, Washington, DC
- Jaurin, B. & Cohen, S. N. (1984) Gene **28**, 83-91
- Jaurin, B. & Cohen, S. N. (1985) Gene **39**, 191-201
- Katz, E., Thompson, C. J. & Hopwood, D. A. (1983) J. Gen. Microbiol. **129**, 2703-2714
- Keddie, R. M. & Jones, D. (1981) in The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria, vol. II (Starr, M. P., Stolp, H., Truper, H. G., Balows, A. & Schlegel, H. G., eds.), pp. 1838-1878, Springer-Verlag, Berlin
- Lampson, B. C. & Parisi, J. T. (1986) J. Bacteriol. **166**, 479-483
- Landick, R., Vaughn, V., Lau, E. T., VanBogelen, R. A., Erickson, J. W. & Neidhardt, F. C. (1984) Cell **38**, 175-182
- Losick, R. & Pero, J. (1981) Cell **25**, 582-584
- McKenny, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C. & Rosenberg, M. (1981) in Gene Amplification and Analysis: Structural Analysis of Nucleic Acids, vol. 2 (Chirikjian, J. G. & Papas, T. S., eds.), pp. 383-415, Elsevier, New York
- Monod, M., Denoya, C. & Dubnau, D. (1986) J. Bacteriol. **167**, 138-147
- Moran, C. P., Jr., Lang, N., LeGrice, S. F. J., Lee, G., Stephens, M., Sonenshine, A. L., Pero, J. & Losick, R. (1982) Mol. Gen. Genet. **186**, 339-346
- Murphy, E. (1985) J. Bacteriol. **162**, 633-640
- Roberts, A. N., Hudson, G. S. & Brenner, S. (1985) Gene **35**, 259-270

- Rosenberg, M. & Court, D. (1979) *Annu. Rev. Genet.* **13**, 319–353
- Schottel, J. L., Bibb, M. J. & Cohen, S. N. (1981) *J. Bacteriol.* **146**, 360–368
- Uchiyama, H. & Weisblum, B. (1985) *Gene* **38**, 103–110
- Walker, M. S. & Walker, J. B. (1970) *J. Biol. Chem.* **245**, 6683–6689
- Ward, J. M., Janssen, G. R., Kieser, T., Bibb, M. J., Buttner, M. J. & Bibb, M. J. (1986) *Mol. Gen. Genet.* **203**, 468–478
- Westpheling, J., Ranes, M. & Losick, R. (1985) *Nature (London)* **313**, 22–27
- Zalacain, M., Gonzalez, A., Guerrero, M. C., Mattaliano, R. J., Malpartida, F. & Jimenez, A. (1986) *Nucleic Acids Res.* **14**, 1565–1581

Received 17 September 1986/12 November 1986; accepted 11 December 1986