Promoter structure and gene function of *Acinetobacter calcoaceticus* encoded *trpFB* operon

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The promoter region of the *Acinetobacter calcoaceticus* encoded *trpFB* operon was determined and found to contain dual promoters. The gene expression of *trpFB* operon was studied as a function of different concentrations of tryptophan and general amino acid starvation. The amounts of mRNA were determined by primer extensions and the expression of transcriptional and translational *lacZ* fusions was studied. The change in the concentration of tryptophan had no effect on transcription or translation, however, a general metabolic effect of increased transcription upon the starvation of all amino acids was detected. It is concluded that *trpFB* is constitutively expressed and lacks regulation.

TRYPTOPHAN biosynthesis from chorismate occurs in all the procaryotes and lower eucaryotes studied so far by the same five reactions[1,2]. However, the structure and organization of these genes encoding the respective enzymes vary considerably[2]. *A. calcoaceticus* contains seven separate genes encoding the enzymes of tryptophan biosynthesis[3]. These genes are located in three unlinked clusters[4]. The nucleotide sequences of *trpGDC*[5], *trpE*[6] and *trpFB*[7,8] have been reported.

The expression of the tryptophan biosynthetic pathway is repressed in many bacteria studied so far in the presence of tryptophan. The molecular mechanisms involved in this regulation of expression are well known for *E. coli*[9,10] and *S. typhimurium*[11]. They involve a tryptophan dependent repressor–operator interaction[12] and attenuation of transcription[9]. The latter mechanism was also found in other enterobacteria[13]. Regulation of the *trp* operon in *B. subtilis* does not occur by conventional attenuation. The terminator and antiterminator structures of mRNA are formed depending upon the concentration of tryptophan in the medium. This requires the presence of a regulatory mRNA binding protein encoded by *mtr* locus[14]. A similar mechanism has also been found for the *B. pumilus* encoded *trp* operon[15,16]. In *Lactococcus lactis*, a similar mechanism as that of *B. subtilis* regulation of *trp* gene expression is proposed[17]. In an archaebacterium, *Methanobacterium thermoautotrophicum* Marburg the *trp* operon is also regulated at the transcriptional level involving a repressor[18]. In *Brevibacterium lactofermentum* a palindromic sequence in the promoter operator region is involved in dual repression–stimulation control of expression of the *trp* operon[19]. While the *trp* genes discussed above are contained in single operons, the regulation of expression of scattered *trp* genes is less well understood. In *Rhizobium meliloti*, of the three gene clusters, only the *trpE* (G) gene is regulated by attenuation[20]. On the contrary, in *Pseudomonas aeruginosa*, the *trpA* and *trpB* encoding tryptophan synthase, are simultaneously induced by indole-glycerol phosphate. This effect is mediated by an activator of transcription[21]. Whereas in *P. mendocina* and *P. marginata,* the *trpB* and *trpA* could not be induced by the indole-glycerol phosphate[22]. In *Caulobacter crescentus*, on the other hand, *trpE* and *trpFBA* genes are constitutively expressed[23]. Further, in *B. subtilis*, an amphibolic *trpG* gene is not regulated transcriptionally, but, at the level of translation[24].

Tryptophan starvation of *A. calcoaceticus trp* auxotrophs resulted in increased levels of all biosynthetic enzymes for this amino acid. The increase was about 5 to 15 fold for the *trpGDC* and *trpE* encoded enzymes, 1.5 to 3 fold for the *trpA* and *trpB* encoded proteins, and 6 fold for *trpF* encoded protein. Only the *trpF* gene product levels were decreased upon the addition of tryptophan to the medium[3,25]. A previous report, however, stated that only the anthranilate synthase levels encoded by *trpE* and *trpG* were affected by the tryptophan level while the other enzymes remained same[26]. *Acinetobacter* is found in environments such as soil or water which are normally subject to considerable variations in substrate composition and temperature. It has been assumed that survival in these different environments required regulation of the metabolism to enable efficient adjustment to changes[27].

In this article, a report on intensive study of the *trpFB* operon expression in dependence of the tryptophan concentration in *A. calcoaceticus* BD413 using primer extension analyses to determine the mRNA levels and *lacZ* fusions to detect the potential regulatory effects on the level of transcription and translation is presented.

Table 1. Bacterial strains and plasmids

	Strain or plasmid	Genotype or markers	Reference
E. coli			
	RRI	F⁻, *hrdS*20 (*r* B⁻, *m*B⁻), *leu*, *ara*, *proA*, *thi*, *lacY*, *galK*, *rpsL* 20, *xyl*, *mtl*, *supE* 44	28
	HB 101	same as RRI except *rec*⁻	38
	JA194 *trpC* 9830	JMB9 r^- m^+, *leu*, *thi*, *trpC (F)*	Gift of C. Yanofsky
	WH202	as RRI except *lacX* 74, *pro*⁺	Gift of A. Wissmann
A. calcoaceticus			
	BD4	Wild type	39
	BD413 *trpE* 27	*trpE*	4
	BD413 *trpB* 18	*trpB*	4
	BD413 *trpA* 23	*trpA*	4
	WH211	BD413 *trpE* 27⁺ (complemented by chromosomal DNA from BD4)	Gift of G. Weins
Plasmid			
	pWH1266	Ap^R, Tc^R	29
	pKOK6	Ap^R, Km^R, Cm^R, *mob*	30
	pWH1754	Ap^R, *trpFB*	7
	pWH1755	Ap^R, (Deletion of *Bss* H2 / *Sal*I fragments from pWH1754)	This work
	pWH1756	Ap^R, Km^R, *lacZ* (Insertion of *Sal*I fragment from pKOK6 in pWH1755)	This work
	pWH1757	Ap^R, *lacZ* (Insertion of *Hin*d III fragment from pKOK6 in pWH1754 after deleting *Bss*H2/*Sal*I fragment and filling the ends and adding *Hin*dIII linker)	This work

Further, evidence is shown for the presence of a weak promoter, P_{F2} in the promoter region of *trpFB*. This weak promoter is functional and more active in *E. coli* than in *A. calcoaceticus*. The results demonstrate that the *trpFB* operon is expressed constitutively under all conditions tested independent of tryptophan concentration in the medium.

Materials and methods

Bacterial strains and plasmids

The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* RR1 *lacZ*ΔM15 (ref. 28) and HB101 were generally used as cloning hosts. *A. calcoaceticus* WH211 was isolated by transformation of *A. calcoaceticus* BD413 *trpE*27 with chromosomal DNA from *A. calcoaceticus* BD4 and selection for growth on minimal medium (Gift from B. Weins). *A. calcoaceticus* BD413 *trpA* 23 (ref. 4) was used for the tryptophan starvation experiments. The plasmids pWH1266 and pWH1274 were used as cloning vectors for *A. calcoaceticus*[29], pKOK6 was used as source for the *lacZ* gene[30] and pWH1754 (ref. 7) contained *trpFB* genes. The plasmid pWH1756 contains a *trpF–lacZ* transcriptional fusion. It was obtained from pWH1754 by digestion with *Bss*H2 and *Sal*I, filling the ends with Klenow DNA polymerase, ligation and transformation to *E. coli* HB101. The resulting plasmid was called pWH1755, prepared, digested with *Sal*I and ligated with *lacZ* containing *Sal*I fragment from pKOK6. The products were transformed to *A. calcoaceticus* WH211 and transformants selected for ampicillin and kanamycin resistance and screened for the blue colonies on X-Gal plates. Candidates were sequenced for their *trpF–lacZ* fusion. pWH1757 contained the *trpF–lacZ* translational fusion and was constructed from pWH1754 by digestion with *Bss*H2 and *Sal*I, filling the protruding ends with Klenow polymerase, ligation with *Hin*dIII linker followed by digestion with *Hin*dIII and ligation with *lacZ* containing *Hin*dIII fragment from pKOK6. The mixture was transformed to *E. coli* WH202 (gift from A. Wissman) and colonies were scored for ampicillin resistance, kanamycin sensitivity and blue colonies on X-Gal plates. The

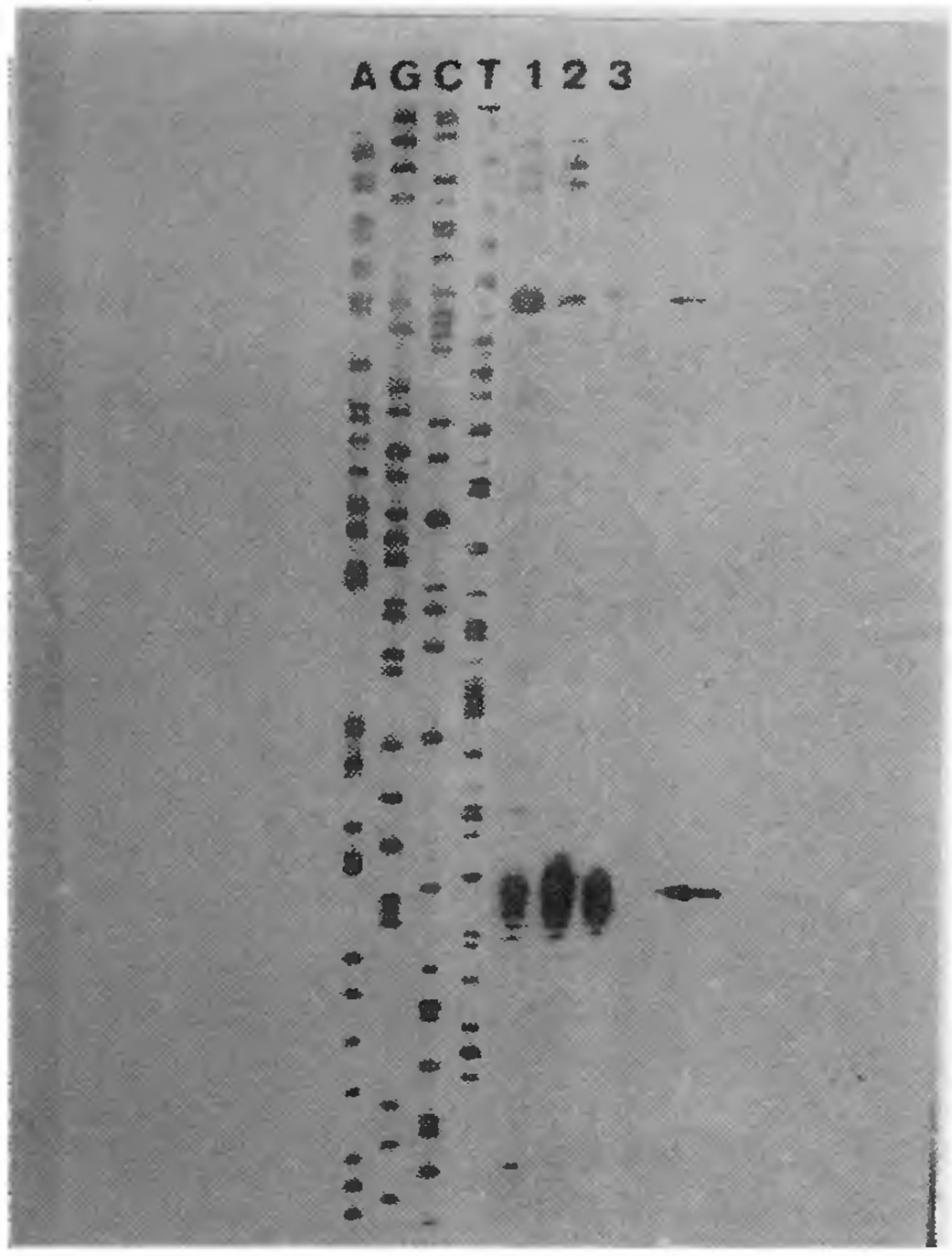

Figure 1. Primer extension analysis of the *trpFB* promoters from *A. calcoaceticus*. *a*, The autoradiograph of the primer extension experiment. Lanes A, G, C and T contain the respective sequencing reactions obtained with the same primer. Lane 1 contains the primer extension products obtained from 1.5 µg total RNA prepared from *E. coli* JA 194 *trp*C9830 transformed with pWH1754 grown in minimal medium. Lane 2 shows the primer extension products from 1.5 µg total RNA prepared from *A. calcoaceticus* BD413 *trpB*18 transformed with pWH1754 grown in minimal medium and lane 3 contains the products of total RNA prepared from *A. calcoaceticus* WH211 grown in minimal medium without casamino acids. The arrows on the right side indicate the bands which are interpreted as transcription start sites. *b*, A sequence interpretation of the experiment. The two promoters P_{F1} and P_{F2} are shown with their consensus sequences, the vertical arrows represent the location of the primer extension products and the horizontal arrow shows the start codon of *trpF*. Furthermore, a palindromic sequence is indicated by the bars below the sequence.

trpF–lacZ translational fusion plasmid was checked by sequencing.

General methods

All general methods were described previously[6,7,29]. The transformation of *A. calcoaceticus* was done as described[29]. Minimal medium contained 1 mM $MgSO_4$, 0.4% glucose and 0.4% casamino acids in M9 salts. For some experiments the casamino acids were omitted as indicated in the text. β-galactosidase activities were determined as published[31]. Preparation of RNA from *Acinetobacter* and primer extension analyses were done as previously described[7,32]. The sequence of the *trpF* specific primer was 5′-CATCTTGGGAACGGGTAATACCGC-3′ and was synthesized using an Applied Biosystems DNA synthesizer. All chemicals for DNA synthesis were obtained from Applied Biosystems, Weiterstadt, Germany. Nucleotide sequencing on double-stranded templates was done by the dideoxy chain termination method[33].

Results and discussion

Organization of promoter region of trpFB

The identification and mapping of the main promoter for the *trpFB* operon in *A. calcoaceticus* has been already described[7]. Figure1 *a* shows a more detailed analysis of transcriptional activity in the sequence upstream of *trpFB* by primer extension. The plasmid and chromosomally encoded RNAs from *Acinetobacter* yielded a strong signal, P_{F1} as described earlier[7] and, in addition, a weak signal, P_{F2} about 79 nucleotides upstream of it. The three bands further upstream occurred only in the plasmid encoded RNA and are, therefore ignored. It is concluded that a second promoter for the *trpFB* operon, which contributed about 5–10% of the total activity, is located upstream of the major promoter. The sequence interpretation in Figure1 *b* shows the locations of promoters in the upstream sequence of *trpFB*. The -35 region of the weak *trpFB* promoter has the sequence TTAACT lacking the highly conserved G at position three of the *E. coli* promoter consensus sequence[34]. Furthermore, it shows a spacing of only 14 base pairs between –35 and –10 regions which is considered unfavourable for promoter activity in *E. coli*[34]. The main promoter contains a spacing of 19 base pairs which is also quite unfavourable. And yet, both promoters are active in *E. coli* as indicated in Figure1 *a*. In this organism, the upstream promoter seems to be more active compared to the main promoter than in *Acinetobacter*. However, other known *Acinetobacter* promoters do not have these unusual spacings between their –10 and –35 regions[35]. Further, the *trpFB* promoter region contained a putative partially palindromic element reminiscent of bacterial operators[7].

Effect of tryptophan concentration on transcription of TrpFB

The effects of increasing concentration on transcription of the *trpFB* operon in *A. calcoaceticus* were studied by primer extension analyses. For that purpose *A. calcoaceticus* WH211 was grown in LB, minimal medium and minimal medium with various concentrations of tryptophan until the $O.D_{600}$ was 1.0. Under these conditions, the cells had a doubling time of 40 min in minimal medium. RNA was isolated from the cells, treated with RNAase-free DNAase I and primer extensions were performed using 25 µg of RNA. The results are shown in Figure 2. No large differences in intensities of the signals were seen, no matter whether the minimal medium contained 0, 2, 5 or 50 µg/ml tryptophan. Even the cells grown in rich medium gave about the same amount of *trpFB* mRNA. Since the minimal medium contained casamino acids, which were described to be free of

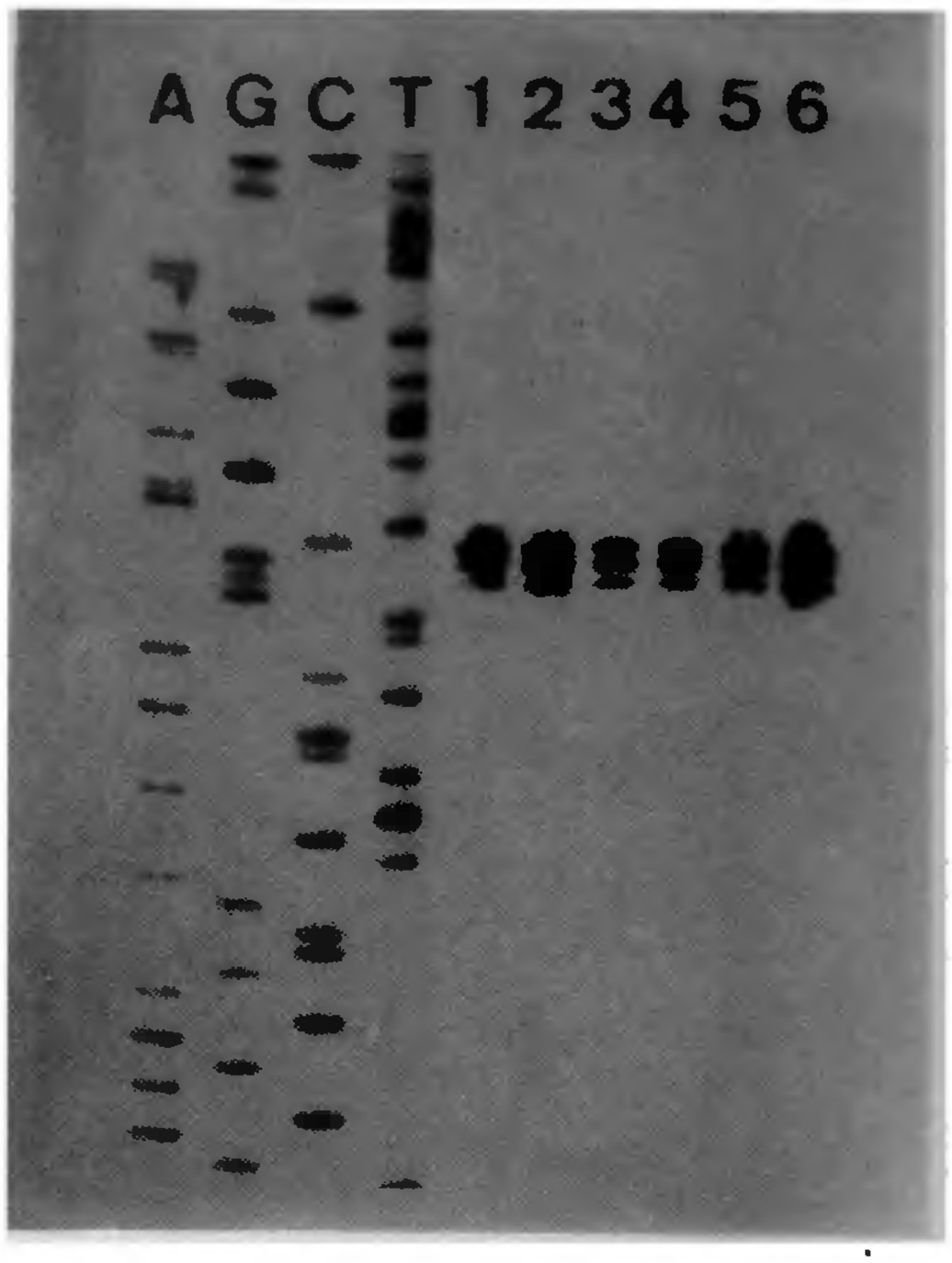

Figure 2. Effect of tryptophan in the growth medium on the transcription of *trpFB* mRNA. The lanes A, G, C and T contain the respective sequencing reactions. Lanes 1 through 6 contain primer extension products of 25 µg total RNA prepared from *A. calcoaceticus* WH211 with the *trpF* specific primer after growth in: Lane 1, LB; lane 2, minimal medium (MM) with 50 µg/ml Trp; lane 3, MM with 5 µg/ml Trp; lane 4, MM with 2 µg/ml Trp; lane 5, MM without TRP; lane 6, MM without casamino acids and Trp.

tryptophan, the amount of *trpFB* mRNA was also determined without this supplement to be sure of omitting any trace of the tryptophan. The result was also shown in Figure 2 and indicated that general amino acid starvation resulted in about 2–3 fold higher mRNA levels. In order to determine whether this was the result of residual tryptophan in the casamino acids, the *trpFB* mRNA of *Acinetobacter calcoaceticus* WH211 grown in the absence of casamino acids was quantified as a function of increasing concentrations of tryptophan. The results are shown in Figure 3. The amounts of *trpFB* mRNA do not depend on the concentration of tryptophan between 0 and 50 µg/ml under these conditions. When compared to the *trpFB* mRNA level in *A. calcoaceticus* grown in LB, the amino acid starvation resulted in a significant increase of *trpFB* transcription. Since this was not dependent on tryptophan but rather on general amino acid starvation, it was interpreted as a result of metabolic regulation[23]. The primer extension experiments were repeated using independent RNA preparations and the results were consistent in all the cases.

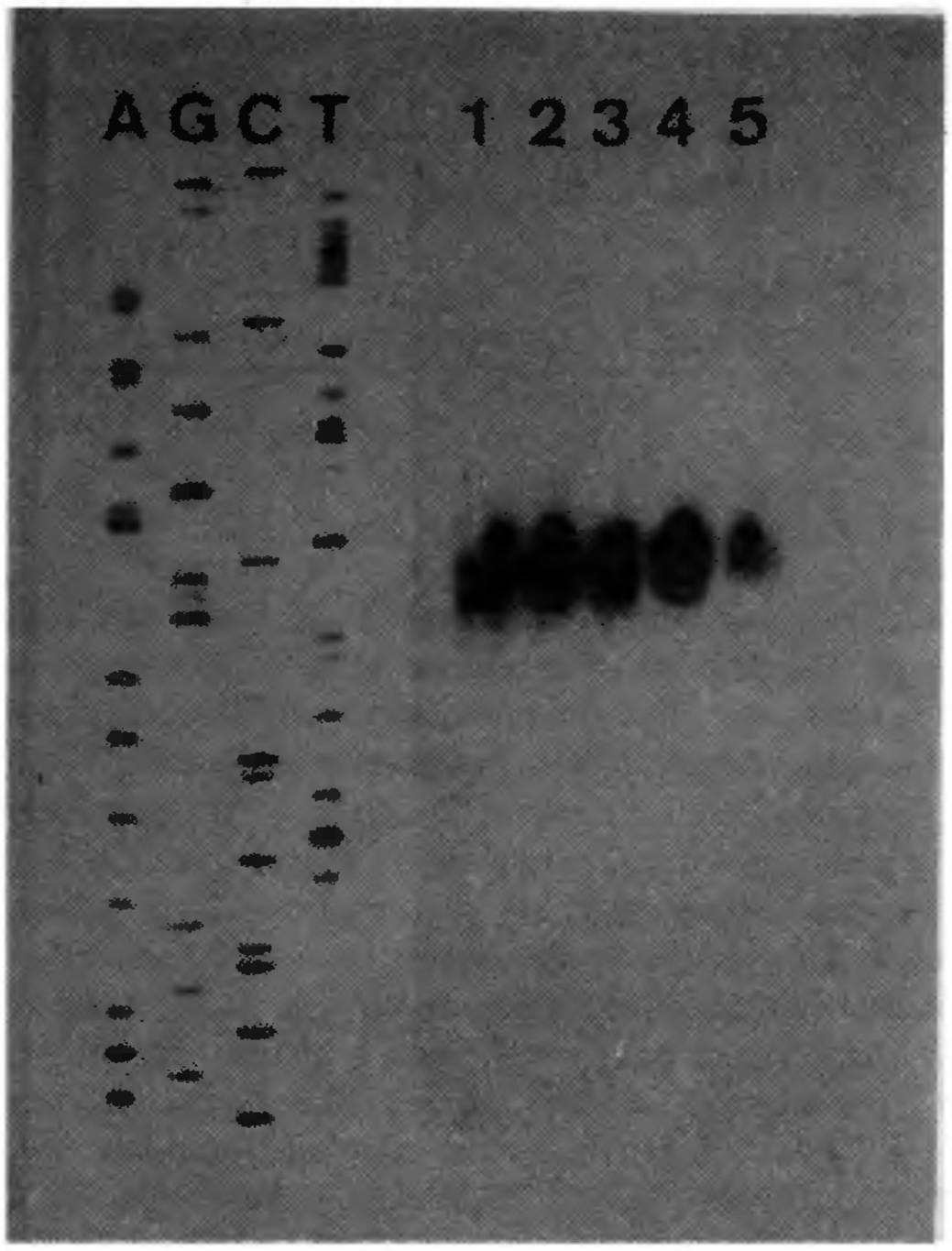

Figure 3. Effect of tryptophan and amino acid starvation on transcription of *trpFB*. The lanes A, G, C and T contain the respective sequencing reactions. Lanes 1 through 5 contain primer extension products obtained from 30 µg total RNA from *A. calcoaceticus* WH211 after growth in: Lane 1; minimal medium (MM) without casamino acids (CAA) and without Trp; lane 2, MM without CAA plus 2 µg/ml Trp; lane 3, MM without CAA with 5 µg/ml Trp; lane 4, MM without CAA with 50 µg/ml Trp; lane 5, LB.

Finally, the possibility of a 'hidden regulation' was explored, as described for *his* operon expression in *S. typhimurium*[36]. In this case a regulation of *his* operon expression could only be seen in *his* auxotrophs under extreme starvation for histidine. *A. calcoaceticus* WH211 and BD413*trpA*23 were grown in LB to an OD_{600} of 0.9. The cells were then rapidly chilled in ice-water, pelleted and one aliquot of cells was used to prepare RNA. The remaining cells were washed in minimal medium without casamino acids and inoculated in minimal medium wihout casamino acids containing 0, 2, and 50 µg/ml tryptophan, respectively. After shaking for 40 min at 30°C, the cells were harvested and RNA was prepared and used for primer extension analyses. The results are shown in Figure 4. Neither the *trp* prototroph nor the *trp* auxotroph showed any dependence of the *trpFB* mRNA amount on the concentration of tryptophan. Therefore, it was concluded that *trpFB* transcription was not affected by tryptophan in the growth medium. In agreement with the results reported by Cohn and Crawford[3], the transcription of *trpFB* seemed to be somewhat higher in the *trp* auxotroph, but the regulation reported by these authors could not be verified here. However, Twarog *et al.*[26] reported that the levels of *trp* enzymes, phosphoribosyl anthranilate isomerase, tryptophan synthase encoded by *trpF* and *trpB* and *trpA* respectively, were not affected in the presence of tryptophan.

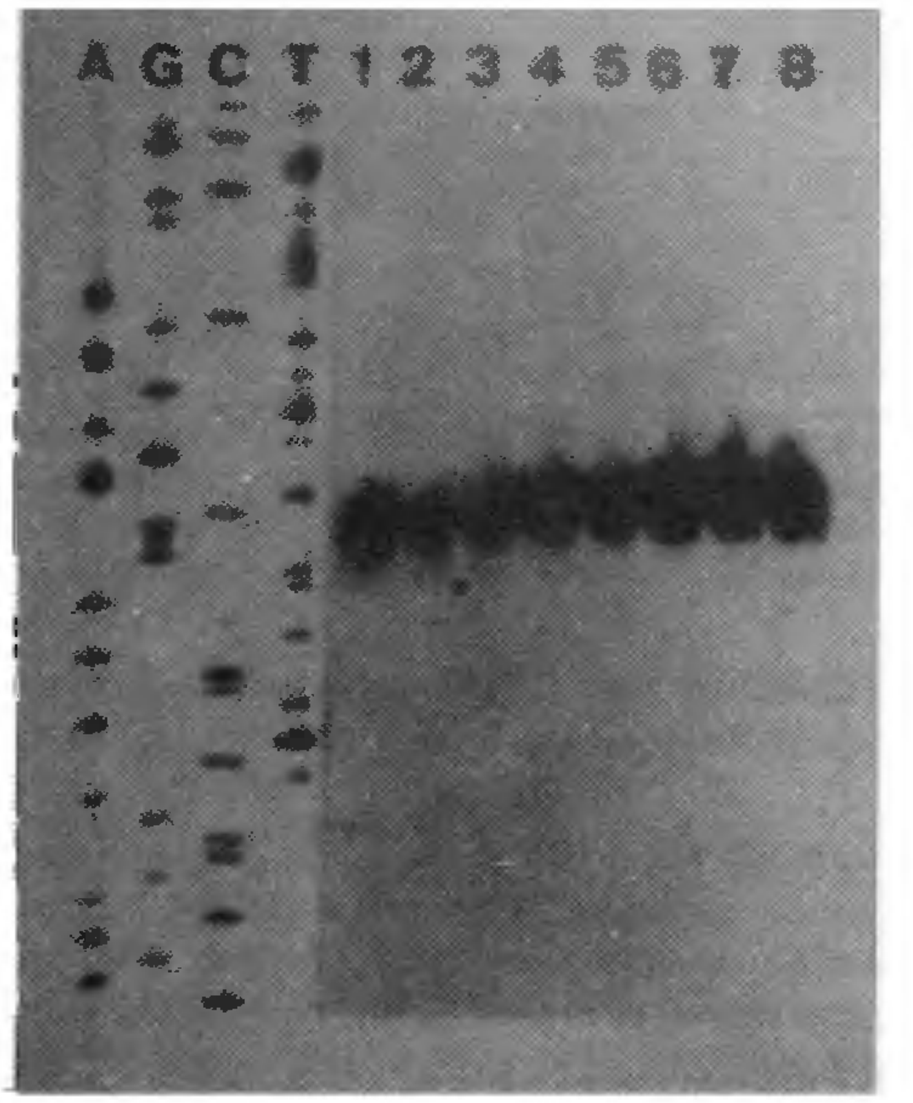

Figure 4. Effect of tryptophan starvation in a *trp* auxotroph on *trpFB* transcription. The lanes A, G, C and T contain the respective sequencing reactions. Lanes 1 through 8 contain primer extension products using a *trpF* specific primer. Lanes 1 through 4 contain the products of 30 µg total RNA prepared from *A. calcoaceticus* WH211 grown in LB (lane 1), followed by a shift in minimal medium (MM) without casamino acids (CAA) (lane 2), in MM without CAA and 2 µg/ml Trp (lane 3), in MM without CAA and 50 µg/ml Trp (lane 4). Lanes 5 through 8 contain the primer extension products obtained from 30 µg of total RNA prepared from *A. calcoaceticus* BD413 *trpA*23 grown under the same conditions, respectively.

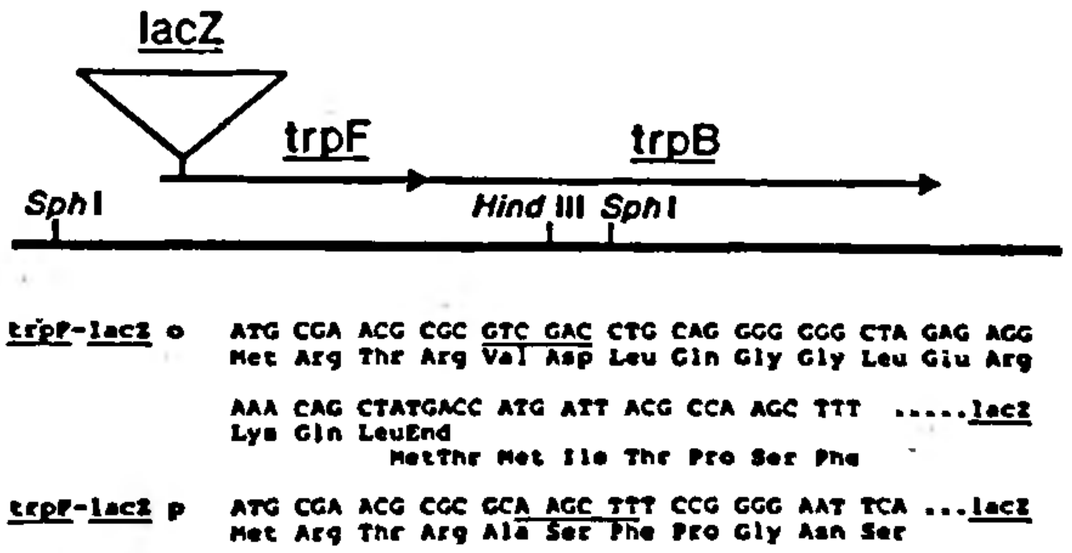

Figure 5. Genetic structure and nucleotide sequence of *trpFB* fusions with *lacZ*. The figure shows the *trpFB* operon with some restriction sites. The location of the *lacZ* fusion is indicated. The nucleotide sequence on the bottom show the operon fusion called *trpF–lacZo* and the protein fusion called *trpF–lacZp*.

Table 2. β-galactosidase expression from *trpF–lacZ* fusions under different growth conditions

A. calcoaceticus strain	Plasmid	β-galactosidase activity in Miller units (U) LB	MM/AP	MM/AP +2 μg/ml Trp	MM/AP + 5 μg/ml Trp	MM/AP +50 μg/ml Trp
WH211	–	No	–	–	–	–
WH211	pWH1755	No	No	No	No	No
WH211	pWH1756	2203 ± 21	1651 ± 22	1616 ± 22	1674 ± 22	1744 ± 23
WH211	pWH 1757	681 ± 21	493 ± 11	493 ± 11	480 ± 11	481 ± 9
WH211	pWH1757*	–	1156 ± 44	1298 ± 22	1333 ± 23	1175 ± 15

U, β-galactosidase activity in Miller units (31) including standard deviation.
*The activity is measured in minimal medium without the addition of casamino acids.
AP, Ampicillin.

Construction and β-galactosidase expression of *trpF–lacZ* fusions in *Acinetobacter*

The transcriptional and translational fusions of *trpF* to *lacZ* were constructed as described earlier. Their genetic organizations and the nucleotide sequences of their fusions are shown in Figure 5. The *trpFB–lacZ* transcriptional fusion contained the first five codons of the *trpF* reading frame followed by eleven codons created by the fusion and the stop codon TGA. The first two nucleotides of the stop codon were part of the *lacZ* ATG start codon. The *trpF–lacZ* translational fusion contained five *trpF* codons. Plasmids with these fusions were transformed to *A. calcoaceticus* WH211, and *A. calcoaceticus* BD413*trpA*23. The resulting strains were grown in LB for overnight cultures. Before inoculation, the cells were pelleted and washed with ice cold minimal medium to remove the residual traces of tryptophan and resuspended in minimal medium. The resultant suspension was inoculated into LB, minimal medium containing 0, 2, 5 and 50 μg/ml of L-tryptophan. The cells were grown for an $O.D_{600}$ 0.6 and tested for the β-galactosidase activities. The results are given in Table 2. While the *A. calcoaceticus* strains used here did not produce any β-galactosidase background activity, all of the fusions directed the expression of *lacZ*. The operon fusion of *trpFB* to *lacZ* resulted around 1700 U with and without the tryptophan in minimal medium; whereas the protein fusions resulted around 480 U. The transcriptional and translational *lacZ* fusion assays under similar conditions differed by a factor around three in all the tested cases. Upon omitting the casamino acids in the minimal medium, the protein fusion assays revealed increased *lacZ* expression about 2.5 fold to around 1160 U, however, addition of tryptophan did not show any regulatory effect, either. Thus, the general metabolic effect of amino acid starvation[37] was also seen on the level of *lacZ* expression, matching the increased transcription of *trp* mRNA mentioned above. The results presented here by *lacZ* assays did not reveal the small regulation as reported earlier, as the multi copy effect of plasmids persisted in the test system. It is interesting to note that the single copy *trpE–lacZ* fusion construct upon integration in chromosome of *A. calcoaceticus* did not show any tryptophan-dependent regulation of *trpE* gene expression (unpublished results, Diploma thesis by S. Schmidt, FAU, Erlangen, Germany, 1992). Further, in the gene regulation of tryptophan biosynthesis, it is the first gene, *trpE* which is regulated in all the known cases. Thus, based on the results presented here, tryptophan-mediated regulation cannot occur at the levels of transcription or translation. Even a hidden regulation in a *trp* auxotroph cannot be found for *A. calcoaceticus* (see Table 2)[36]. It is therefore concluded that the *trpFB* genes are constitutively expressed in this organism. In the case of *Caulobacter crescentus*[23], *trpE* and *trpFBA* genes were not regulated and it was argued that the natural habitats of that strain were usually poor in amino acid supply, and regulation of expression of genes for amino acid biosynthesis would be wasteful. And the same argument could certainly hold true for *A. calcoaceticus* as well.

1. Crawford, I. P., *Bacteriol. Rev.*, 1975, **39**, 87–120.
2. Crawford, I. P., *Annu. Rev., Microbiol.*, 1989, **43**, 567–600.
3. Cohn, W. and Crawford, I. P., *J. Bacteriol.*, 1976, **127**, 367–379.
4. Sawula, R. V. and Crawford, I. P., *J. Bacteriol.*, 1972, **112**, 795–805.
5. Kaplan, J. B., Goncharoff, P., Seibold, A. M. and Nichols, B. P., *Mol. Biol. Evol.*, 1984, **1**, 456–472.
6. Haspel, G., Hunger, M., Schmucker, R. and Hillen, W., *Mol. Gen. Genet.*, 1990, **220**, 475–477.
7. Kishan, V. and Hillen, W., *J. Bacteriol.*, 1990, **172**, 6151–6155.
8. Ross, C. M., Kaplan, J. B., Winkler, M. E. and Nichols, B. P., *Mol. Biol. Evol.*, 1990, **7**, 74–81.
9. Yanofsky, C., *Nature*, 1981, **289**, 751–758.
10. Yanofsky, C., *Mol. Biol. Evol.*, 1984, **1**, 143–161.
11. Yanofsky, C. and Crawford, I. P., in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (eds Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. and Umbarger, H. E.), American Society for Microbiology, Washington DC, 1987, vol. 2, pp. 1453–1472.
12. Platt, T., in *Genetics of Bacteria* (eds Scaife, J., Leach, D. and Galizzi, A.), Academic Press, London, 1985, pp. 255–278.
13. Blumenberg, M. and Yanofsky, C., *J. Bacteriol.*, 1982, **152**, 49–56.
14. Shimotsu, H., Kuroda, M. I., Yanofsky, C. and Henner, D. J., *J. Bacteriol.*, 1986, **166**, 461–471.

15. Kuroda, M. I., Shimotsu, H., Henner, D. J. and Yanofsky, C., *J. Bacteriol.*, 1986, **167**, 792–798.
16. Kuroda, M. I., Henner, D. and Yanofsky, C., *J. Bacteriol.*, 1988, **170**, 3080–3088.
17. Bardowski, J., Ehrlich, S. D. and Chopin, A., *J. Bacteriol.*, 1992, **174**, 6563–6570.
18. Gast, D. A., Jenal, U., Wasserfallen, A. and Leisinger, T., *J. Bacteriol.*, 1994, **176**, 4590–4596.
19. Guerrero, C., Matees, L. M. and Malumbress, M., *Gene*, 1994, **138**, 35–41.
20. Bae, Y. M. and Crawford, I. P., *J. Bacteriol.*, 1990, **172**, 3318–3327.
21. Chang, M., Hadero, A. and Crawford, I. P., *J. Bacteriol.*, 1989, **171**, 172–183.
22. Olekhnovich, I. N., Kotik, E. A. and Iuk Fomichov, *Genetika*, 1992, **28**, 31–37.
23. Ross, C. M. and Winkler, M.E., *J. Bacteriol.*, 1988, **170**, 769–774.
24. Slock, J., Stahly, D. P., Han, C. Y., Six, E. W. and Crawford, I. P., *J. Bacteriol.*, 1990, **172**, 7211–7226.
25. Sawula, R. V. and Crawford, I. P., *J. Biol. Chem.*, 1973, **248**, 3573–3581.
26. Twarog, R. and Liggins, G. L., *J. Bacteriol.*, 1970, **104**, 254–263.
27. Juni, E., *Annu. Rev. Microbiol.*, 1978, **32**, 349–371.
28. Bolivar, F., Rodriguez, R. L., Greener, P. J., Betlach, M. C., Heynecker, H. L., Boyer, H. W., Crosa, J. H. and Falkow, S., *Gene*, 1977, **2**, 95–133.
29. Hunger, M., Schmucker, R., Kishan, V. and Hillen, W., *Gene*, 1990, **87**, 45–51.
30. Kokotek, W. and Lotz, W., *Gene*, 1989, **84**, 467–471.
31. Miller, J. H., in *Experiments in Molecular Genetics*, Cold Spring Harbor, New York, 1972.
32. Chirgwin, J. M., Przybyla, A. E., Mac Donald, R. J. and Rutter, W. J., *Biochemistry*, 1979, **18**, 5294–5299.
33. Sanger, F., Nicklen, S. and Coulson, A. R., *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 5463–5467.
34. Hawley, D. K. and Mc Clure, W. R., *Nucleic Acids Res.*, 1983, **11**, 2237–2255.
35. Ehrt, S., Ornston, L. N. and Hillen, W., *J. Bacteriol.*, 1994, **176**, 3493–3499.
36. Winkler, M. E., in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (eds Neidhardt, F. C., Ingraham, J. L., Low, K. B., Megasanik, B., Schaechter, M. and Umbarger, H. E.), American Society for Microbiology, Washington DC, 1987, vol. 1, pp. 395–411.
37. Rose, J. K. and Yanofsky, C., *J. Mol. Biol.*, 1972, **69**, 103–118.
38. Bolivar, F. and Backmaun, K., *Methods Enzymol.*, 1979, **68**, 245.
39. Juni, E., *J. Bacteriol.*, 1972, **112**, 917–931.

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Date: 19–22 December 1998
Place: Dibrugarh

The Wadia Institute of Himalayan Geology is organizing this Group Discussion in collaboration with the Department of Applied Geology, Dibrugarh University. It includes two days' deliberations at Dibrugarh University, followed by two days field excursion along the Brahmaputra Valley.

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Tenth International Conference on Rapidly Quenched and Metastable Materials

Date: 23–27 August 1999
Place: Bangalore

The topics include: Materials preparation, Phase stability, Cluster structure and evolution, Undercooling, Nanochemistry, Properties (Mechanical, electrical, magnetic, chemical), Quasicrystals, High strength low density alloys

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