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Revised interpretation of the sequence containing the *murE* gene encoding the UDP-*N*-acetylmuramyl-tripeptide synthetase of *Escherichia coli*

The *murE* gene encoding the UDP-*N*-acetylmuramyl tripeptide synthetase of *Escherichia coli* is located immediately downstream of the *pbpB* gene [1,2]. The region extending downstream of *pbpB* was first partially sequenced by Nakamura *et al.* [1] and recently totally sequenced by Tao & Ishiguro [3]. We have now also sequenced this same open reading frame and established an identical sequence. Tao & Ishiguro [3] tentatively assigned the ATG codon at position 179–181 (Fig. 1) as the initiation codon. However, we have determined the *N*-terminal amino acid sequence of the purified *murE* gene product being: A-D-D/R-N-L-R-D-L-L-A-P-?-V-P-D-A-P-S. Assuming the post-translational loss of a methionine residue, this *N*-terminal amino acid sequence is in agreement with an initiation codon GTG at positions 107–109 located 72 nucleotides upstream from the previously assigned ATG initiation codon. The presence of a TGA codon at positions 83–85 in the same frame as the *murE* coding region excludes any other possibility. In *E. coli* GTG is an unusual initiation codon encountered in approx. 8% of cases [4]. This initiation codon is preceded by a sequence (underlined in Fig. 1) matching the consensus sequence for a ribosome binding site [5]. Furthermore, the two consensus sequences, ATTAAT at positions 85–90 and TGACA at positions 56–60, clearly indicate that *murE* has an efficient independent promoter. This confirms previous work [2] which also showed that the *murF* gene immediately downstream of *murE* was under the control of the *murE* promoter.

The coding region for *murE* thus overlaps the end of the *pbpB* coding region by 11 base pairs and contains 1485 nucleotides which are translated into 495 amino acids corresponding to a protein with a molecular mass of 53382. This value is in agreement with that of the protein expressed by the maxicell technique [2,6]. The synthetase activity requires ATP hydrolysis and, as previously observed with the *murD* and *murF* gene products [7,8], a domain A characteristic of ATP-binding proteins was found between amino acids 116 and 121 (underlined in Fig. 1). The excess of the Glu + Asp content (63 residues) over the Arg + Lys

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     F  G  A  I  M  G  G  V  L  R  T  M  N  I  E  P  D  A  L  T
  1 TTTGGTGCCATCATGGGCGGCGTATTGCGTACCATGAACATCGAGCCGGATGCGCTGACA

     T  G  D  K  N  E  F  V  I  N  Q  G  E  G  T  G  G  R  S
 61 ACGGGCGATAAAAATGAATTTGTGATTAATCAAGGCGAGGGGACAGGTGGCAGATCGTAAT
                                          M  A  D  R  N

122 TTGCGCGACCTTCTTGCTCCGTGGGTGCCAGACGCACCTTCGCGAGCACTGCGAGAGATG
     L  R  D  L  L  A  P  W  V  P  D  A  P  S  R  A  L  R  E  M

182 ACACTCGACAGCCGTGTGGCTGCGGCGGGCGATCTCTTTGTAGCTGTAGTAGGTCATCAG
     T  L  D  S  R  V  A  A  A  G  D  L  F  V  A  V  V  G  H  Q

242 GCGGACGGGCGTCGATATATCCCGCAGGCGATAGCGCAAGGTGTGGCTGCCATTATTGCA
     A  D  G  R  R  Y  I  P  Q  A  I  A  Q  G  V  A  A  I  I  A

302 GAGGCGAAAGATGAGGCGACCGATGGTGAAATCCGTGAAATGCACGGCGTACCGGTCATC
     E  A  K  D  E  A  T  D  G  E  I  R  E  M  H  G  V  P  V  I

362 TATCTCAGCCAGCTCAACGAGCGTTTATCTGCACTGGCGGGCCGCTTTTACCATGAACCC
     Y  L  S  Q  L  N  E  R  L  S  A  L  A  G  R  F  Y  H  E  P

422 TCTGACAATTTACGTCTCGTGGGCGTAACGGGCACCAACGGCAAAACCACGACTACC...
     S  D  N  L  R  L  V  G  V  T  G  T  N  G  K  T  T  T  T
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Fig. 1. Overlapping between the end of the *pbpB* sequence and the beginning of the *murE* sequence

The end of the coding region of the *pbpB* gene extends upstream from position 117. The beginning of the coding region of the *murE* gene extends downstream from position 107. A consensus sequence for a ribosome-binding site is located at positions 97 to 100 (underlined). A domain A characteristic of ATP-binding proteins is located at positions 452–469 (underlined).

content (45 residues) is consistent with the low value of the isoelectric point determined for the purified protein.

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1. Nakamura, M., Maruyama, I. N., Soma, M., Kato, J., Suzuki, H. & Hirota, Y. (1983) Mol. Gen. Genet. **191**, 1–9
2. Mengin-Lecreuix, D., Parquet, C., Desviat, L. R., Pla, J., Flouret, B., Ayala, J. A. & van Heijenoort, J. (1989) J. Bacteriol. **171**, 6126–6134
3. Tao, J. S. & Ishiguro, E. E. (1989) Can. J. Microbiol. **36**, 1051–1054
4. Gold, L. & Stormo, G. (1987) in *Escherichia coli* and *Salmonella typhimurium*, Cellular and Molecular Biology (Neidhardt, F. C., ed), vol. 2, pp. 1302–1307, American Society for Microbiology, Washington DC
5. Hershey, J. W. B. (1987) in *Escherichia coli* and *Salmonella typhimurium*, Cellular and Molecular Biology (Neidhardt, F. C., ed), vol. 1, pp. 613–647, American Society for Microbiology, Washington DC
6. Maruyama, I. N., Yamamoto, A. H. & Hirota, Y. (1988) J. Bacteriol. **170**, 3786–3788
7. Parquet, C., Flouret, B., Mengin-Lecreulx, D. & van Heijenoort, J. (1989) Nucleic Acids Res. **17**, 5379
8. Mengin-Lecreuix, D. & van Heijenoort, J. (1990) Nucleic Acids Res. **18**, 183

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Myelin basic protein does not contain a phosphatidylinositol anchor

Phosphatidylinositol (PI) linkage to proteins has been identifed as a mechanism by which proteins can be anchored to lipid bilayers (Low, 1987). In a report by Yang *et al.* (1986) a PI-anchor was described in bovine myelin basic protein (BMBP). However, Smith *et al.* (1987) were unable to confirm the data of Yang *et al.* (1986). They found that the inositol content of BMBP, prepared by four different methods, varied from 1 mol/1000 mol of BMBP to 1 mol/10000 mol of BMBP. At the time of the report of Smith *et al.* (1987), we had completed similar studies with human myelin basic protein (HMBP) and were unable to demonstrate that HMBP contained a PI anchor. Since the report of Yang *et al.* (1986) continues to be cited (Hudson *et al.*, 1989), we report those aspects of our studies which complement the studies of Smith *et al.* (1987) so that this matter may be laid to rest.

Non-specific binding of PI to HMBP was determined by two methods. Radioactive phosphatidylinositol 4,5-bisphosphate ([^{32}P]PIP_2), was added to purified human myelin and to isolated HMBP in separate experiments. HMBP was isolated from purified myelin by our procedure (Lowden *et al.*, 1966) and by that of Yang *et al.* (1986). The HMBP fraction contained 1.3×10^5 c.p.m. After SDS/PAGE, the HMBP cut from the gel contained 26 c.p.m. When purified HMBP was incubated with [^{32}P]PIP_2 and subjected to SDS/PAGE, 59 c.p.m. were recovered with HMBP (6.7×10^5 c.p.m. were applied to the gel). Therefore, non-specific binding of PI to HMBP was ruled out.

Freshly prepared human myelin was incubated with [^{32}P]ATP as described by Yang *et al.* (1986), the HMBP was isolated, treated with trypsin and carboxypeptidase Y and the peptides resolved on a C_{18} reversed phase column (Waters Associates). Whereas Yang *et al.* (1986) recovered a lipophilic peptide in the 100% methanol eluant we were unable to detect any peptide in this eluant. An alternative method in which the ^{32}P-labelled HMBP was digested with endoproteinase LysC to generate peptides also failed to yield a radioactive lipophilic peptide.

Table 1. Gas chromatography–mass spectroscopy of hydrolysed and non-hydrolysed human myelin basic protein

Each sample was hydrolysed in 6 M-HCl at 110 °C for 24 h in the presence of the internal standard, deuterated *myo*-[$^2H_2$]inositol (MSD Isotopes, Montreal, Canada). After several lyophilization cycles following the addition of water each time, the residue was reacted with 5% trimethylsilyl chloride/45% NO-(bistrimethylsilyl) trifluoroacetamide in anhydrous pyridine (Sigma Chemical Co.) for 3 h *in vacuo* at room temperature. Samples were analysed by g.c.–m.s. using electron impact and selected ion monitoring (Sherman *et al.*, 1977).

Method preparation of HMBP	*m/z* 305	*m/z* 307	Relative 307/305 (%)	Inositol (ng)	HMBP (μg)
(*a*) SDS/PAGE					
Hydrolysed	12745	9191	72.1	138.6	250
Non-hydrolysed	196145	139753	71.0	140.8	1300
(*b*) Acid-extracted, hydrolysed	152395	109774	72.0	138.8	1300

Neither partial acid hydrolysis of [^{32}P]HMBP in 1 M-HCl for 30 min at 37 °C as described by Yang *et al.* (1986), nor digestion with PI-specific phospholipase C (gift from M. G. Low, Columbia University, New York) yielded any of diphospho-, triphosphoinositides or diacylglycerol. Neither galactosamine nor ethanolamine were detected in acid hydrolysates of HMBP (6 M-HCl for 4 h at 100 °C for amino sugars and 6 M-HCl for 24 h at 100 °C for ethanolamine). Release of [^{32}P]P_i by acid phosphatase from peptides isolated by h.p.l.c. from a LysC digest suggested phosphorylation occurred at seryl and threonyl residues (J. Ramwani & M. A. Moscarello, unpublished work).

G. c.–m. s. was used to detect inositol in three preparations of HMBP: (*a*) hydrolysed SDS/PAGE-prepared HMBP, (*b*) hydrolysed acid-extracted MBP and (*c*) unhydrolysed HMBP. The data are summarized in Table 1. The ratio of inositol (*m/z* 305) to deuterated inositol (*m/z* 307) was 71–72%. Since no additional inositol was generated as a result of acid hydrolysis, the presence of a PI anchor was unlikely.

In both the reports of Yang *et al.* (1986) and Smith *et al.* (1987), unfractionated BMBP was used. At alkaline pH, MBP can be fractionated into seven or eight components or charge isomers, differing by the net positive charge (Wood & Moscarello, 1989), the result of post-translational modifications. The presence of a PI anchor on one of the charge isomers could be missed by using the unfractionated protein. The *m/z* 307/305 ratio was 71% for all charge isomers tested. We concluded that a PI anchor was not a post-translational modification of any of the HMBP charge isomers.

In conclusion, our results confirm those reported by Smith *et al.* (1987) that HMBP, like BMBP, does not contain a PI-anchor. Furthermore, isolation of individual charge isomers, which might be expected to yield a species enriched in this particular post-translational modification, failed to identify a HMBP species with a PI anchor.

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Hudson, L., Fredrich, V. L., Behar, T., Dubois-Dalcq, N. & Lazzarini, R. A. (1989) J. Cell Biol. **109**, 717–727