Multiple Roles for the Twin Arginine Leader Sequence of Dimethyl Sulfoxide Reductase of Escherichia coli*

Received for publication, November 17, 1999, and in revised form, March 3, 2000 Published, JBC Papers in Press, May 8, 2000, DOI 10.1074/jbc.M909289199

Damaraju Sambasivarao, Raymond J. Turner‡, Joanne L. Simala-Grant§, Gillian Shaw, Jing Hu¶, and Joel H. Weiner*

From the Medical Research Council Group in Molecular Biology of Membrane Proteins, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

Dimethyl sulfoxide (Me₂SO) reductase of Escherichia coli is a terminal electron transport chain enzyme that is expressed under anaerobic growth conditions and is required for anaerobic growth with Me₂SO as the terminal electron acceptor. The trimeric enzyme is composed of a membrane extrinsic catalytic dimer (DmsAB) and a membrane intrinsic anchor (DmsC). The amino terminus of DmsA has a leader sequence with a twin arginine motif that targets DmsAB to the membrane via a novel Sec-independent mechanism termed MTT for membrane targeting and translocation. We demonstrate that the Met-1 present upstream of the twin arginine motif serves as the correct translational start site. The leader is essential for the expression of DmsA, stability of the DmsAB dimer, and membrane targeting of the reductase holoenzyme. Mutation of arginine 17 to aspartate abolished membrane targeting. The reductase was labile in the holoenzyme. These mutants failed to support growth on glycerol-Me₂SO minimal medium. Replacing the DmsA leader with the TorA leader of trimethylamine N-oxide reductase produced a membrane-bound DmsABC with greatly reduced enzyme activity and inefficient anaerobic respiration indicating that the twin arginine leaders may play specific roles in the assembly of redox enzymes.

Recently, we and others have reported the discovery of a novel system, which targets and translocates folded, cofactor-containing proteins to and across the cytoplasmic membrane of bacteria (1–4). A similar system was previously identified in chloroplasts and some mitochondria (5). This system, named MTT (for membrane targeting and translocation) or TAT (for twin arginine translocation), recognizes an amino-terminal motif, (S/T)RR...N. The MTT leader sequence is typically 30–60 amino acids long, contains a conserved twin arginine motif, (S/T)RRXFX/ILK, and has been found to be associated with a large number of periplasmic redox proteins (6, 7). Although the role of this signal sequence in protein export was originally proposed for the periplasmic hydrogenase of Desulfovibrio vulgaris (8), understanding of this unique leader had to await the discovery of the MTT genes.

One of the twin Arg leader proteins identified in a data base search was the catalytic subunit of Me₂SO reductase (6). This enzyme is a heterotrimer, composed of a molybdopterin guanine dinucleotide containing a catalytic subunit (DmsA, 85.8 kDa), an iron-sulfur subunit (DmsB, 23.1 kDa), and a membrane intrinsic subunit (DmsC, 30.8 kDa) (9, 10). The DmsA and -B subunits function as a catalytic dimer, whereas DmsC is the membrane anchor subunit and binds menaquinone (11). It was argued by Berks (6) that DmsA together with DmsB could be targeted for export via the MTT export pathway. However, extensive biochemical, immunological, electron microscopic, and electron paramagnetic resonance studies have been carried out on the topological organization of the Me₂SO reductase (12, 13). The two membrane extrinsic subunits, DmsA and -B were shown to face the cytoplasmic side of the membrane, contrary to the periplasmic localization of most other twin Arg leader-containing proteins.

The twin Arg motif of DmsA was not recognized in the initial cloning and sequencing of the dms operon (9). N-terminal analysis of the purified DmsA subunit revealed that the mature, purified protein began with the sequence Val-Asp-Ser... (see Fig. 1) and that the first upstream Met (16 residues upstream, M30 in Fig. 1) was proposed as the initiating Met (9). However, it now appears that DmsA may actually initiate at a Met, 46 residues upstream of the Val-46 (Fig. 1). This 45-amino acid leader encodes a twin Arg motif and has Ala-His-Ala immediately upstream of the cleavage site, in accord with other proteins containing this type of leader (6, 14).

The electron transfer properties of the Me₂SO reductase in Escherichia coli are well documented (15, 16). However, very little is known about the role of the leader peptide. In the present study, we confirm that DmsA does indeed initiate at the more upstream Met (which we have now designated as M1, Fig. 1) and that the first upstream Met is required for anaerobic growth with Me₂SO as the terminal electron acceptor. The reductase holoenzyme. These mutants failed to support growth on glycerol-Me₂SO minimal medium.

* This work was funded in part by the Medical Research Council of Canada. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ Present address: Structural Biology Group, Dept. of Biological Sciences, University of Calgary, Alberta, Canada T2N 1N4.
§ Present address: Dept. of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.
¶ Recipient of a summer student fellowship from the Alberta Heritage Foundation for Medical Research.
¶ To whom correspondence should be addressed: Tel.: 780-429-2761; Fax: 780-429-0886; E-mail: joel.weiner@ualberta.ca.
1 The abbreviations used are: MTT, for membrane targeting and translocation; or TAT for twin arginine translocation; G-F medium, glycerol-fumarate minimal medium; Me₂SO, dimethyl sulfoxide; G-D medium, glycerol-Me₂SO minimal medium; PAGE, polyacrylamide gel electrophoresis; bp, base pair(s); PCR, polymerase chain reaction; BV, benzyl viologen; TMAO, trimethylamine N-oxide.
2 This paper is available on line at http://www.jbc.org

EXPERIMENTAL PROCEDURES

Materials—Oligonucleotides were purchased from the DNA core facility, Department of Biochemistry, University of Alberta, Edmonton, Canada or Life Technologies, Inc., Canada. All molecular biology reagents were purchased from Life Technologies Inc. The Sculptrcor in vitro mutagenesis kit was obtained from Amersham Pharmacia Biotech. ECL Western blotting detection reagents were purchased from Amer...
Twin Arginine Leader of Me$_2$SO Reductase

All constructs were verified by restriction digestion analysis of the mini DNA preparations and DNA sequencing reactions to confirm the mutations or deletions in the dms operon.

Media and Growth Conditions—Aerobic overnight bacterial cultures were grown at 37 °C unless indicated otherwise, in Luria-Bertani (LB) medium. Overnight bacterial cultures were grown in LB medium (20 ml) containing 2.5 µg/ml of chloramphenicol. A 1% inoculum was used from these overnight cultures for all experiments, unless indicated otherwise. Anaerobic growth experiments in glycerol minimal media were carried out in 160-ml screw capped conical flasks with a Klett tube attachment (Klett flasks) for direct monitoring of the bacterial growth as a function of time. The minimal medium was supplemented with 5% glucose, 2% yeast extract, vitamin B1, terminal electron acceptor, ammonium monoborate, and antibiotics, as described (11). Me$_2$SO served as the terminal electron acceptor for the growth experiments in the Klett flasks.

For the measurement of Me$_2$SO reductase activity, the cells were grown anaerobically in 1000-ml conical flasks at 37 °C for 48 h on glycerol-fumarate minimal medium (G-F medium), for 24 h in peptone-fumarate medium, or for 8 h in Terrific broth (TB) medium (21). Me$_2$SO reductase was expressed constitutively under the above anaerobic growth conditions. Cells were harvested and treated as described earlier for the preparation of the membrane and supernatant (periplasm plus cytoplasm) fractions (11).

**Gel Electrophoresis and Western Blotting**—Proteins were fractionated on a 7.5% or a 12% resolving acrylamide gel using a Bio-Rad mini slab gel apparatus with an SDS buffer system (23). Proteins from SDS-polyacrylamide gel electrophoresis (PAGE) gels were transferred to nitrocellulose filters and probed with the anti-DmsA and anti-DmsB antibodies as described (11). Western blot analysis of whole cells was performed essentially as above, except the cell pellets were directly lysed in the SDS solubilization buffer and heated in a boiling water bath for 2 min before loading on SDS-PAGE gels.

**TT Expression Studies**—Strain K38/pGP1-2, carrying pPDMS223-encoding dmsABC or various mutants, was grown overnight in LB medium at 30 °C. A 5% inoculum of this culture was transferred into fresh LB medium (6.0 ml) and grown for 2 h, and a 1.0-ml aliquot was drawn for the zero time sample. To the remaining 5.0 ml of bacterial culture, was added 2.5 µl fresh LB medium (5.0 ml) at 54 °C, to facilitate rapid temperature equilibration of the culture to 42 °C. The incubation was continued for another 15 min in a water bath set at 42 °C to induce T7 RNA polymerase. Rifampicin (250 µg/ml of culture), an inhibitor of chromosomally encoded RNA polymerase, was added, and the incubation was continued at 42 °C for 15 min. All subsequent incubations were carried out at 37 °C (20). Aliquots (1.0 ml) were drawn at various time points and spread on LB medium with the appropriate antibiotic.

**Enzyme Assays—**DmsABC dimer enzyme assays were carried out by following the substrate trimethylamine N-oxide (TMAO)-dependent oxidation of the reduced benzyl viologen (BV) (11, 24). DmsABC holoenzyme assays were carried out by monitoring the oxidation of the menaquinol analogue, lapachol, as described earlier (25).

**Analytical Techniques—**DNA sequencing was carried out using an Applied Biosystems model 373A DNA sequencer at the DNA core facility in the Department of Biochemistry, University of Alberta, Edmonton, Canada. All the data reported here represent at least two independent experiments, and the results from a representative experiment are presented.

**RESULTS**

**Investigation of the Translational Start Site of the DmsA Leader Sequence and Its Role in Expression of DmsAB Polypeptides**—When the dmsABC sequence was reported (9, 10), we proposed that DmsA initiated at a methionine, 16 residues upstream of a valine, the first amino acid of the purified DmsA subunit (M30 in Fig. 1). Subsequent bioinformatic analysis of the dmsABC sequence by Altschul (6) suggested that DmsA actually initiated at a methionine, which is 45 residues upstream of the start codon (M1, Fig. 1) and contained a consensus twin Arginine motif. To determine if M1 was the initiating Met, we mutated this start codon to the stop codon TAA, and expressed the construct (pT7M1 Stop) using a T7 promoter system. Following expression, the proteins were probed by Western blotting with...
anti-DmsA and anti-DmsB antibodies. As shown in Fig. 2, vector pT718R did not express DmsAB, whereas pDMS223 (wild-type dmsABC) showed good expression of the DmsAB subunits. The DmsAB polypeptides were observed as early as 30 min and peaked at 2 h. The expression from the pT7M1 Stop plasmid showed that the intensity of the DmsA polypeptide was drastically reduced with limited expression at 30 and 60 min followed by rapid degradation. Expression of the DmsB polypeptide was relatively unaffected, even up to 21 h. These results indicated that DmsA had a long 45-amino acid leader, which contained a consensus twin arginine motif, and that an internal Met-30 could not serve as an efficient initiating methionine (Fig. 1). Under these experimental conditions, DmsB could not protect the truncated form of DmsA originating at the putative second initiating Met-30 in the pT7M1 Stop construct (Fig. 1) from degradation.

Role of the Conserved Arginine in the DmsA Leader Sequence—The two arginines of the twin Arg consensus sequence have been shown to be highly conserved within the family of polypeptides exhibiting the motif (Fig. 1, R16 and R17). These residues have been shown to be important for the expression and/or the function of the polypeptides that rely on the MTI system for protein targeting and transport (14, 26, 27). We mutated Arg-17 in the DmsA leader to an Asp (R17D) and examined the effect on the expression and stability of DmsA in the T7 system as described above (Fig. 2). Western blot analysis revealed that DmsA was expressed to levels comparable to the wild-type, and the amount of DmsA was only slightly reduced at 21 h, compared with the wild-type (Fig. 2; pT7R17D). These results indicate that the R17D mutation has no adverse effects on the expression or stability of the DmsA polypeptides under the conditions of the T7 expression and analysis of the whole cell lysates by Western blotting. The expression of the DmsC polypeptide could not be analyzed in these experiments due to the lack of the corresponding antibody (12).

FIG. 1. Signal sequence of the DmsA subunit. The wild-type DmsA signal sequence is shown for plasmid pDMS160 and for the mutants listed under “Experimental Procedures.” The twin Arg motif is boxed and the consensus sequence is shown at the bottom. Amino acid changes, or additions resulting from the cloning manipulations, are shown in lowercase letters. The gaps in the leader sequences are introduced to facilitate alignment of the constructs. The start of mature DmsA at position 46 is shown by an arrow. The AXA motif, which is conserved among the twin arginine leader sequences and may be the recognition site for the protease involved in the processing of the signal sequence is underlined. Amino acids deleted following the M1 in pTDMS10 and pDMS189 are represented by dashed lines. The stop codon in pDMSM1 Stop is shown by the asterisk. The lac promoter construct pDMS190 carries the entire dms operon, similar to pDMS160, except for the amino acids introduced as a result of engineering the restriction sites. The TorA leader replaces the Dms leader in pDMS191 and contains several amino acids derived from the mature TorA identified by italics. Construction of the plasmids is described under “Experimental Procedures.”

FIG. 2. The twin arginine signal sequence is essential for expression and stability of DmsA. The expression studies were carried out in the T7-promoter vector pT718R and the wild-type (pDMS223) and mutant DmsA leader sequences (pT7M1 Stop and pT7R17D). These plasmids are described under “Experimental Procedures.” The DmsA and DmsB subunits are indicated.
FIG. 3. **The twin arginine signal sequence of DmsA is essential for anaerobic growth of E. coli on G-D medium.** Bacterial growth measured as Klott units are plotted as a function of time (hours). The recombinant plasmids in E. coli DSS301 were tested for growth with either the native anaerobic promoter (A) or the lac promoter (B). The plasmids are identified in the figure. Individual data points for the experiments using the plasmids pDMS10, pDMSR17D, pDMSM1 Stop, and DSS301 were omitted for clarity, because these plasmids did not show any growth over 36 h. Individual growth profiles for the mutants pDMSA43N and pDMSA43N:A45N were identical, and representative data for pDMSA43N:A45N are shown.

We investigated replacement of the dms leader with the TorA leader of inducible TMAO reductase. We used lac promoter constructs, pDMS189, pDMS190, and pDMS191 to facilitate the interpretation of the leader substitution (or deletions) while keeping the promoter background constant. The TorA leader has a twin Arg motif and translocation of TMAO reductase to the periplasm is dependent on the MTT system (2, 3). Plasmid pDMS191, with a torA leader in place of the dmsA leader, showed poor growth (Fig. 3B). These studies indicate that an intact dmsA leader was essential for the production of functional enzyme. Examination of the control lac promoter constructs (Fig. 3B) indicated that pDMS189, lacking a dmsA leader, could not support growth, similar to its parent pTDMS10 (Fig. 3A). The wild-type lac promoter-dmsA leader construct (pDMS190) supported good growth even in the absence of added inducer.

The Role of the DmsA Leader on the Targeting, Assembly, and Activity of Me₂SO Reductase—To probe the enzymatic activity of the leader mutants described above, we measured the Me₂SO-dependent oxidation of benzyl viologen (BV) as a measure of DmsAB function and lapachol-catalyzed reduction of Me₂SO as a measure of the DmsABC in the membrane and soluble fractions from E. coli DSS301 cells grown on minimal glycerol-fumarate medium (Table I). Me₂SO reductase activity was localized predominantly to the membrane fraction in cells harboring pDMS160, pDMS190, and the cleavage site mutants pDMSA43N and pDMSA43N:A45N. The activity data correlated well with the anaerobic growth experiments (Fig. 3).

Membrane vesicles from E. coli strain DSS301/pDMSM1 Stop expressed very low levels of BV and lapachol activities. The BV activity observed was predominantly in the soluble fraction (Table I), in agreement with the growth measurements. Similarly, there was no detectable BV or lapachol enzyme activity when the leader was deleted (pTDMS10). In this mutant, the leader was replaced with a multicloning site that encodes 11 amino acids, including an initiating Met at the amino terminus of DmsA unrelated to the DmsA leader (Fig. 1). These studies indicate that the leader sequence is an absolute requirement to sustain the anaerobic growth, enzyme activity, stability, and localization of the Me₂SO reductase holoenzyme (Fig. 3 and Table I).

Strain DSS301/pDMSR17D with a mutation in the second Arg of the twin Arg motif expressed DmsA (Fig. 4B), but this enzyme did not associate with the membrane and only very minimal levels of enzyme activity were detected in the soluble fraction. Although nearly normal levels of DmsA appear to accumulate following T7 expression (Fig. 2), we have found that under the standard growth conditions (peptone-fumarate medium for 24 h or on glycerol-fumarate medium for 48 h; data not shown), the precursor form of DmsA is degraded. As will be shown below (Fig. 4B), mutant precursor enzyme from pDMSR17D was poorly processed to the mature form.

We have examined the expression of dmsABC using the lac promoter construct (pDMS190). In minimal medium pDMS190 expressed similar total activity units in the absence of an inducer (isopropyl-1-thio-β-D-galactopyranoside) compared with pDMS160. The membrane distribution of the BV and lapachol activities was also similar to pDMS160 (Table I). We also compared the activity profiles of the various constructs grown on rich medium (TB) for 8 h (Table II). For the wild-type pDMS190, 97% of the activity was found in the membrane fraction.

E. coli/pDMS189, lacking a leader, had only 6% of the wild-type activity, and 76% of this activity was soluble. DSS301/pDMS191 with a TorA leader in place of the DmsA leader, had about 20% of the wild-type activity, and 90% was membrane-bound, corroborating the slow anaerobic growth seen on glycerol-Me₂SO medium. These results indicate that the twin arginine leader is required for stability and for the association of DmsAB with the membrane.

Western Blot Analysis of the DmsA Subunit from the Wild-type and Signal Sequence Mutants—To confirm the role of the leader in membrane targeting and stability of DmsA, we used Western blot analysis of membrane and soluble fractions from cells harboring various plasmids encoding Me₂SO reductase. The distribution of DmsA with the wild-type and mutant leaders was examined from cells grown anaerobically on G-F medium for 48 h to corroborate the activity measurements summarized in Table I. DmsA expressed by DSS301/pDMS160, pDMS190, pDMSA43N, and the double mutant pDMSA43N:A45N was observed predominantly in the membrane fraction (Fig. 4A). The distribution of DmsA reflected the distribution of specific activity (Table I). Cells harboring plasmids pDMSM1 Stop (Fig. 4A) and pTDMS10 (data not shown) showed no immunoreactive material in either the membrane or soluble fractions.

Analysis of the DmsA from DSS301/pDMSR17D mutant was carried out from cells grown on TB medium for 8 h (Fig. 4B). Cells grown on G-F or peptone-fumarate media did not show any immunoreactive DmsA presumably due to degradation (data not shown). However, analysis of the cell fractions derived from cells grown in rich medium revealed three immuno-
Twin Arginine Leader of Me₂SO Reductase

Comparison of the Me₂SO reductase activity from *E. coli* DSS301 carrying wild-type and mutant DmsA leader sequences. Bacterial cultures (1 liter) were grown anaerobically in glycerol-fumarate minimal medium for 48 h at 37 °C. Enzyme activities were measured using either reduced benzyl viologen (A) or lapachol (B) as the electron donor and TMAO as the electron acceptor. The total units of activity (micromoles of benzyl viologen or lapachol oxidized per min) and the specific activity (expressed as units/mg of protein) in the membrane and soluble fractions are indicated.

| Plasmid     | Total units Membrane | Soluble Total units Membrane |
|-------------|----------------------|-------------------------------|
| pDMS160     | 593                  | 25.0 (93)*                    | 0.49 (7) 27.0 |
| pDMS190     | 520                  | 19.0 (94)                     | 0.43 (6) 26.0 |
| pDMSA43N    | 630                  | 27.0 (92)                     | 0.6 (8) 34.0 |
| pDMSA43N    | 778                  | 27.0 (94)                     | 0.66 (6) 39.0 |
| pDMSR17D    | 43                   | 0.20 (13)                     | 0.5 (87) ND |
| pDMSM1 Stop | 5                    | 0.2 (100)                     | ND ND ND |
| pTDMS10     | ND                   | ND                            | ND ND ND |

* Numbers in parentheses, percentages, distribution of activity in the membrane and soluble fractions.

![Figure 4: Western blot analysis of the DmsA subunit from strains expressing the wild-type and mutant enzymes](image)

**A** Western blot analysis of the DmsA subunit from strains expressing the wild-type and mutant enzymes. Recombinant plasmids in *E. coli* DSS301 are identified above each lane. See “Experimental Procedures” for a full description of the plasmids. The membrane (m) and soluble (s) fractions were separated on 7.5% SDS-PAGE gels, transferred to nitrocellulose, and blotted for the DmsA subunit. The precursor (P), mature (M), and degraded (D) forms of the DmsA subunit are identified. DMSO STD, purified Me₂SO reductase. A, data from the cells grown on glycerol-fumarate medium for 48 h. B, data from the cells grown on TB medium for 8 h.

**B** Western blot analysis of the DmsA subunit under control of Lac promoter

*E. coli* DSS301, carrying the various recombinant plasmids, was grown anaerobically in TB medium for 8 h at 37 °C. Assay of the reductase activity and calculation of the total units, specific activity, and distribution of the activity in membrane and soluble fractions were as described in Table I.

**Table II**

Comparison of Me₂SO reductase activity under control of Lac promoter

| Plasmid | Total units | Me₂SO reductase specific activity |
|---------|-------------|----------------------------------|
|         |             | Membrane Soluble                  |
| pDMS189 | 35          | 0.20 (24)* 0.20 (76)              |
| pDMS190 | 519         | 11.0 (97) 0.10 (3)                |
| pDMS191 | 111         | 2.10 (90) 0.13 (10)               |

* Numbers in parentheses indicate %.

Discussion

The MTT system plays an essential role in the translocation of several folded, cofactor-containing, redox proteins to the periplasm (1–3). These proteins have long amino-terminal leader sequences with a twin Arg motif and are usually soluble or bound to the periplasmic side of the membrane. The DmABC and FdoGHI enzymes are membrane-bound, multimeric subunit proteins, and the DmABC and FdoG subunits have similar twin Arg signal peptides. However, a great deal of experimental evidence has shown that DmABC and FdoG subunits face the cytoplasmic side of the membrane and are not translocated across the membrane (12, 28). We have proposed that the MTT system is needed to associate the DmABC subunits with the membrane anchor subunit, DmC (1, 11).

In this study we provide experimental evidence for the presence of a 45-residue twin Arg leader on the DmABC subunit. We also show that the leader is essential for the stability and function of the holoenzyme. This contrasts with the periplasmic TMAO and Me₂SO reductases from *E. coli* and *Rhodobacter sphaeroides* (2, 29). In these enzymes, deletion of the twin Arg leader resulted in cytoplasmic localization of the enzyme, consistent with the predicted role of the leader peptide (2, 3, 29); however, the leaderless enzymes were stable and incorporated the molybdate cofactor.

The DmABC leader has two methionine residues, Met-1 and Met-30 (Fig. 1). Met-30 was found to be a poor initiation site and led to minimal synthesis of the reductase (Fig. 2). This Met is not conserved in DmABC homologues (6). The confirmation of a 45-residue leader necessitates renumbering of amino acid residues in DmABC. Previous publications have assumed that DmABC initiated at Met-30, based on the original sequence study (9), and the first residue of the mature protein, Val46, was previously numbered Val-16.

Mutagenesis studies of the conserved Arg, within the twin Arg motif, were performed on the [Ni-Fe] hydrogenase from *D*.
Twin Arginine Leader of Me₂SO Reductase

Weiner, J. H., Blouos, P. T., Shaw, G. M., Lubitz, S. P., Frost, L., Thomas, G. H., Cole, J. A., and Turner, R. J. (1998) Cell 93, 93–101

REFERENCES

1. Weiner, J. H., Blouos, P. T., Shaw, G. M., Lubitz, S. P., Frost, L., Thomas, G. H., Cole, J. A., and Turner, R. J. (1998) Cell 93, 93–101
2. Santini, C. L., Ize, B., Chanal, A., Muller, M., Giordano, G., and Wu, L. F. (1998) EMBO J. 17, 101–112
3. Sargent, F., Bogsch, E. G., Stanley, N. R., Wexler, M., Robinson, C., Berks, B. C., and Palmer, T. (1996) EMBO J. 17, 3640–3650
4. Segawa, H., Sato, A., Shibata, S., and Murata, K. (1997) Science 278, 1446–1470
5. Bogsch, E. G., Sargent, F., Stanley, N. R., Berks, B. C., Robinson, C., and Palmer, T. (1998) J. Biol. Chem. 273, 18003–18006
6. Berks, B. C. (1996) Mol. Microbiol. 22, 393–404
7. Fekkes, P., and Driessen, A. J. (1999) Microbiol. Mol. Biol. Rev. 63, 161–173
8. Dongen, W. V., Hagen, W., Berg, W. V. D., and Veeger, C. (1988) FEBS Lett. 250, 5–9
9. Bilous, P. T., Cole, S. T., Anderson, W. F., and Weiner, J. H. (1988) Mol. Microbiol. 2, 765–769
10. Bilous, P. T., and Weiner, J. H. (1988) J. Bacteriol. 170, 1511–1518
11. Sambasivarao, D., and Weiner, J. H. (1991) J. Bacteriol. 173, 5935–5943
12. Sambasivarao, D., Scabra, D. G., Triebel, C., and Weiner, J. H. (1996) J. Bacteriol. 172, 5932–5948
13. Rothery, R. A., and Weiner, J. H. (1993) Biochemistry 32, 5855–5861
14. Niviere, V., Wong, S. L., and Voordouw, G. (1992) J. Gen. Microbiol. 138, 2173–2183
15. Rothery, R. A., and Weiner, J. H. (1991) Biochemistry 30, 8296–8305
16. Weiner, J. H., Rothery, R. A., Sambasivarao, D., and Triebel, C. A. (1992) Biochim. Biophys. Acta 1102, 1–16
17. Triebel, C. A., Rothery, R. A., and Weiner, J. H. (1996) J. Biol. Chem. 271, 4620–4626
18. Rothery, R. A., and Weiner, J. H. (1996) Biochemistry 35, 3247–3257
19. Tatar, S., and Richardson, C. C. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 1074–1078
20. Latour, D. J., and Weiner, J. H. (1989) Biochem. Cell Biol. 67, 251–259
21. Schober, J., Fritsch, E. R., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
22. Zhang, Y. B., and Broome-Smith, J. K. (1990) Gene 96, 51–57
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Sambasivarao, D., and Weiner, J. H. (1991) Curr. Microbiol. 23, 105–110
25. Rothery, R. A., Chatterjee, J., Kiema, G., McDermott, M. T., and Weiner, J. H. (1990) Biochem. J. 262, 35–41
26. Haltig, D., Hou, B., Freudi, R., Sprenger, G. A., and Klosgen, R. B. (1999) FEBS Lett. 447, 95–98
27. Brink, S., Bogsch, E. G., Edwards, W. R., Hynds, P. J., and Robinson, C. (1998) FEBS Lett. 434, 425–430
28. Benoït, S., Abaibou, H., and Mandraud-Berthelot, M. A. (1998) J. Bacteriol. 180, 6025–6034
29. Fekkes, P., and Driessen, A. J. (1998) Mol. Microbiol. 30, 434–436
30. Halbig, D., Hou, B., Freund, R., Sprenger, G. A., and Klosgen, R. B. (1999) FEBS Lett. 447, 95–98
31. Fekkes, P., and Driessen, A. J. (1999) Microbiol. Mol. Biol. Rev. 63, 161–173
32. Fekkes, P., and Driessen, A. J. (1999) Microbiol. Mol. Biol. Rev. 63, 161–173
33. D. Sambasivarao, H. A. Dawson, J. Hu, and J. H. Weiner, manuscript in preparation.