Membrane Topology of the Transposon 10-encoded Metal-Tetracycline/H\(^+\) Antiporter as Studied by Site-directed Chemical Labeling*  

Tomomi Kimura**, Masae Ohnuna**, Tetsuo Sawai**, and Akihito Yamaguchi***  

From the \(\Delta\)Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, Mihogaoka, Ibaraki, Osaka 567, the \(\Delta\)Faculty of Pharmaceutical Sciences, Osaka University, Yamadaoka, Suita, Osaka 565, and \(\Delta\)Division of Microbial Chemistry, Faculty of Pharmaceutical Sciences, Chiba University, Chiba 263, Japan

The transposon (Tn) 10-encoded metal-tetracycline/H\(^+\) antiporter (Tn10-TetA) is predicted to have a membrane topology involving 12 transmembrane domains on the basis of the hydropathy profile of its sequence and the results of limited proteolysis; however, the experimental results of limited proteolysis are not enough to confirm the topology because proteases cannot gain access from the periplasmic side (Eckert, B., and Beck, C. F. (1989) J. Biol. Chem. 264, 11663–11670). One or two cysteine residues were introduced into each predicted hydrophilic loop or the N-terminal segment of Tn10-TetA by site-directed mutagenesis, and then the topology of the protein was determined by examining whether labeling of the introduced Cys residue by membrane-permeant \(\text{[14C]N-ethylmaleimide (}[14\text{C}]\text{NEM})\) was prevented by preincubation of intact cells with the membrane-impermeant maleimide, 4-acetamido-4'-maleimidystilbene-2,2'-disulfonic acid (AMS). The binding of \([14\text{C}]\text{NEM}\) to the S36C (loop 1–2), L97C (loop 3–4), S156C (loop 5–6), R238C (loop 7–8), S296C (loop 9–10), S65C (loop 2–3), D120C (loop 4–5), S199C and S201C (loop 6–7), T270C (loop 8–9), and S328C (loop 10–11) mutants was not affected by pretreatment with AMS, indicating that these residues are located on the periplasmic surface. In contrast, \([14\text{C}]\text{NEM}\) binding to the S4C (N-terminal segment), S65C (loop 2–3), D120C (loop 4–5), S199C and S201C (loop 6–7), T270C (loop 8–9), and S328C (loop 10–11) mutants was affected by pretreatment with AMS, indicating that these residues are on the cytoplasmic surface. These results for the first time thoroughly confirm the 12-transmembrane topology of the metal-tetracycline/H\(^+\) antiporter.

Tn10-TetA is regarded as a member of the major facilitator family including uniporters and symporters because of the structural similarity and the conserved sequence motif, DRRXGR (9). Tn10-TetA is important for elucidating the common structural and functional features of the major facilitator family because it is the only antiporter in this family of which the molecular mechanism of the transport has been extensively studied in detail. Using inside-out membrane vesicles, it has been shown that Tn10-TetA mediates the 1:1 antiport of a divalent cation-tetracycline complex with a proton (4, 10). Site-directed mutagenesis studies on Tn10-TetA revealed the presence of some functionally essential aspartic acid residues (11, 12). One of these aspartic acid residues is located in the sequence motif common to the major facilitator family transporters (13). The functional and/or structural importance of the aspartic acid conserved in this motif was also confirmed in \(\alpha\)-ketoglutarate permease (14) and lactose permease (15), suggesting a common molecular mechanism for symporters and antiporters.

Most major facilitator family transporters are predicted to contain 12 membrane-spanning domains (9). As to tetracycline/H\(^+\) antiporters, Eckert and Beck (1), and Henderson and Maiden (16) proposed similar 12-transmembrane segment models for the membrane topologies of Tn10-TetA and pBR322-TetA, respectively, based on the results of hydropathy analysis. The model of Tn10-TetA was partially confirmed by biochemical and immunological studies. (i) 4 of the 5 predicted cytoplasmic loops were cleaved by the proteases used from the inner side of the membrane (1). (ii) The N-terminal methionine is accessible for cyanate modification in inside-out vesicles but not in spheroplasts (1). (iii) The anti-C-terminal peptide antibody could bind to Tn10-TetA only from the inner side of the membrane (17). However, there is no biochemical evidence for the periplasmic location of any of the predicted periplasmic segments of Tn10-TetA, because it is resistant to protease digestion from the periplasmic side of the membrane (1). Allard and Bertrand (18) studied the membrane topology of pBR322-TetA, which is a tetracycline-resistant protein encoded by pBR322, by means of alkaline phosphatase (PhoA) gene fusions. With this technique, they succeeded in determining the periplasmic location of three of the six predicted periplasmic loops, whereas the fusion proteins connected at the predicted periplasmic loop 1–2, loop 3–4, and loop 9–10 showed unexpectedly low enzyme activity (18). Since the hydropathy plot of Tn10-TetA (1) showed no distinct hydrophilic segment between TM3 and TM4, or TM9 and TM10, the periplasmic location of loop 3–4 and loop 9–10 was not supported by either the biochemical evidence or the predictive algorithm.

Site-directed chemical labeling of cysteine residues with a

---

* This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan and a Grant-in-Aid from the Chiba-Geigy Foundation for the Promotion of Science. 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.

** Research Fellow of the Japan Society for the Promotion of Science.

† To whom correspondence should be addressed: Tel.: 81-6-879-8545; Fax: 81-6-879-8549; E-mail, akihito@sanken.osaka-u.ac.jp.

The abbreviations used are: Tn, transposon; AMS, 4-acetamido-4'-maleimidystilbene-2,2'-disulfonic acid; MOPS, 3-morpholinopropanesulfonic acid; NEM, N-ethylmaleimide; TM, transmembrane.
Membrane Topology of the Tetracycline/H\(^+\) Antipporter

suflhydryl reagent is useful for analyzing the topology of a polytopic membrane protein (19). Tn10-TetaA contains only one cysteine residue at position 377; however, SH reagents did not bind to Cys-377 due to it being buried in the hydrophobic interior of the membrane (20). There was no difference in \[^{14}\text{C}]\text{NEM} binding between the wild-type and the C377A (Cys-less) mutant (30). Therefore, the reactivity of an SH reagent to a mutant TetaA protein represents the reactivity of the reagent to an introduced cysteine residue (20). In this study, we constructed 15 site-directed mutants of Tn10-TetaA in which cysteine residues were introduced into putative periplasmic or cytoplasmic loop regions, followed by chemical labeling with the membrane-permeant SH reagent, \[^{14}\text{C}]\text{N}-\text{ethylmaleimide}, after preincubation with or without a membrane-impermeant SH reagent to determine their sidedness.

**EXPERIMENTAL PROCEDURES**

**Materials—**N-[\text{Ethyl}-\text{L}-\text{14C}]maleimide (1.5 GBq/mmol) was purchased from DuPont NEN. 4-Acetamido-4’-maleimidostilbene-2,2’-disulfonic acid (AMS) was from Molecular Probes Inc. All other materials were of reagent grade and obtained from commercial sources.

**Bacterial Strains and Plasmids—**Escherichia coli CJ236 (21), TG1 (22), and W3104 (23) were used for the preparation of single-stranded DNA, transformation after mutagenesis, and inverted vesicle preparation, respectively. E. coli JM109 (21) and BMH71-18 mutS (21) were used for oligonucleotide-directed mutagenesis by the Kunkel method (21). pC71183 (24) and pLG72 (11) are plasmids carrying 2.45-kilobase Tn10-tetA and tetR gene fragments cloned into pUC18 (purchased from Takara, Kyoto, Japan) and pLG339 (25), respectively. A multi-copy plasmid, pCT1183, was used for mutagenesis, and a low copy number plasmid, pLG2T2, was used for expression of the mutant tet gene.

**Site-directed Mutagenesis—**Cysteine mutants were constructed by oligonucleotide-directed site-specific mutagenesis according to the method of Kunkel (21) using pC71183 as a template, and a synthetic oligonucleotide containing mismatched base pairs was used for generating a new restriction site in order to check the mutation. Mutations were at first detected as the appearance of a newly produced restriction site and then verified by DNA sequencing using a Shimadzu DNA sequencer DSQ-1000. The new restriction site was then inserted into the appropriate E. coli host and verified by DNA sequencing.

**Site-directed Mutant TetA Proteins and Prevention by Preincubation with 4-Acetyl-L-maleimide—**First of all, we examined the binding of \[^{14}\text{C}]\text{NEM} to the S65C and L97C mutants of Tn10-TetaA in intact cells or everted membrane vesicles after preincubation in the presence or absence of 5 mM AMS for 30 min at 30 °C, followed by incubation with 0.5 mM \[^{14}\text{C}]\text{NEM} for 5 min. The reaction was stopped by dilution with the same buffer containing excess unlabeled NEM. The cells were disrupted by brief sonication. The disrupted cells or everted vesicles were solubilized, and the TetaA proteins were precipitated using anti-C-terminal peptide antiserum and Pansorbin \text{S. aureus} cells (28). The radioactive bands on SDS electrophoresis gels of these precipitates were visualized with a BAS-1000 Bio-Imaging Analyzer (Fuji Film Co., Tokyo, Japan). The symbols, + and −, under the lanes indicate the presence or absence of AMS during the preincubation, respectively.

**RESULTS**

**[^14C]N-Ethylmaleimide Binding to the S65C and L97C Mutant TetA Proteins and Prevention by Preincubation with 4-Acetamido-4’-maleimidostilbene-2,2’-disulfonic Acid—**First of all, we examined the binding of \[^{14}\text{C}]\text{NEM} to the S65C and L97C mutants of Tn10-TetaA in intact cells or everted membrane vesicles after preincubation in the presence or absence of AMS. Ser-65 and Leu-97 are located in predicted cytoplasmic loop 2–3 and periplasmic loop 3–4, respectively. Ser-65 is important for the transport function, but position 65 is a unique hot spot in loop 2–3 for inactivation by an SH reagent (28). Leu-97 is a hot spot in loop 3–4 similar to position 65.2 It is certain that the S65C and L97C mutants retain the wild-type conformation of TetaA, because they showed wild-type drug resistance. A binding experiment was performed as described under “Experimental Procedures.” Since AMS is a membrane-permeant reagent, both the S65C and L97C mutants were equally labeled by \[^{14}\text{C}]\text{NEM} in intact cells or everted membrane vesicles in the absence of AMS (Fig. 1), indicating that these positions are exposed to the aqueous phase. Under these conditions containing 0.5 mM AMS, about 90% of the tetracycline transport activity was abolished (data not shown), indicating that the labeling reaction was almost saturated. With 1.0 mM AMS, the degree of inactivation no longer increased, whereas 0.2 mM AMS abolished only 65% activity. The degree of binding of AMS to the S65C mutant was not affected by preincubation with a membrane-impermeant reagent, AMS, in intact cells, whereas the binding of AMS in everted membrane vesicles was prevented by pretreatment with 5 mM AMS (Fig. 1), indicating that position 65 is located inside of intact cells and exposed to the outside medium in everted membrane vesicles. This confirms the cytoplasmic location of position 65. In contrast, the binding of \[^{14}\text{C}]\text{NEM} to the L97C mutant was completely prevented by preincubation with 5 mM AMS for 30 min in intact cells (Fig. 1), indicating that position 97 is located on the outside surface of the cyto-

---

2 A. Yamaguchi, Y. Shiina, T. Kimura, and T. Sawai, manuscript in preparation.
plasmic membrane. With 2 mM AMS, the degree of protection decreased to about half, while various preincubation times, from 5 to 30 min, did not have a significant effect on the degree of protection. The binding of NEM to the L97C mutant was partially blocked in everted membrane vesicles (Fig. 1), probably due to contamination by right-side-out or unsealed membrane vesicles. These results clearly showed that an experiment on the binding of [14C]NEM to a cysteine mutant and the protection by AMS is useful for determining whether a position is located on the cytoplasmic or periplasmic surface.

Construction and Activity of Cysteine Mutants—We constructed 15 different cysteine mutants to determine the sidedness of the hydrophilic loop regions of Tn10-tetA. In addition to positions 65 and 97, cysteine residues were introduced into positions 4, 36, 120, 156, 199, 201, 238, 270, 296, 328, 357, 360, and 365 using the mutagenic oligonucleotide primers listed in Table I. The locations of the mutations are depicted in Fig. 2. These positions covered all of the 11 putative hydrophilic loop regions and the N-terminal segment. The cytoplasmic location of the C-terminal is evident because the anti-C-terminal peptide antibody is only accessible from the inner side of the membrane (17). Mutagenesis was performed using pCT1183 as a template, and the sequences were determined by DNA sequencing, followed by transfer of the mutant tetA gene to a low copy number plasmid, pLGT2, by fragment exchange. No additional unexpected mutations occurred within the fragment transferred to pLGT2. The expression of the mutant TetA proteins was detected by 1) immunoblotting using anti-C-terminal peptide antiserum, and 2) Coomassie Brilliant Blue staining of gels after SDS electrophoresis of the immunoprecipitated TetA proteins. The degrees of expression of the TetA proteins were not significantly affected by these mutations (data not shown).

At first, the tetracycline resistance levels of E. coli W3104 cells harboring these mutant plasmids were measured on agar plates. As shown in Table II, 10 of the 15 mutants showed tetracycline resistance comparable with that of the wild type. S156C, S201C, and R238C showed moderate resistance, indicating that these mutations did not cause any significant alteration in the protein conformation. In contrast, the D120C and Y357C mutants had almost completely lost the drug resistance (Table II). As to the D120C mutant, it retained comparable tetracycline transport activity to that of the wild type when the activity was measured in everted membrane vesicles (Fig. 3). A similar discrepancy between transport activity and the resistance level was also observed for the D120N mutant (12), of which minimum inhibitory concentration was 19 μg/ml but the transport activity was comparable with that in the wild type. This discrepancy may be based on alteration of the kinetic

### Table I

| Mutagenic primer | Primer sequence | Codon change |
|------------------|-----------------|--------------|
| S4C              | 5’ CTTTGATACAGCTTGTCATTTC-3’ | TCG → TGT |
| S38C             | 5’-AGCGATATCTTTCCATGCAAAATTCACG-3’ | TCG → TGC |
| S97C             | 5’-ACGCCAGAGTACACATCCAAGC-3’ | CTG → TGC |
| D120C            | 5’ CGTCATTGCAGTGCACCTCAAG-3’ | GAT → TGC |
| S156C            | 5’-ATC GGACAGATCTCCTGTC-3’ | TCA → TGT |
| S201C            | 5’-GATGTATACACAATCGATTG-3’ (disappearance of EcoRI) | TCG → TGT |
| R238C            | 5’-CATCCGAACATCTTGCTT-3’ | CGT → TGT |
| S296C            | 5’-TTCACATATAAGCCCAAAAGC-3’ | TCT → TGT |
| S328C            | 5’ GATAGGCGCTTGCTGATGACACTTT-3’ | AGT → TGT |
| Y347C            | 5’-ATGATTACATAGACGATAAA-3’ | TAT → TGT |
| S360C            | 5’-ATCCCATATGGTATAGACACTT-3’ | TCA → TGT |
| D365C            | 5’-CAGCACACCATATGGTAGT-3’ | GAT → TGT |
Thus, some of the conformational change must be caused by the Asp-120 mutation. However, as judged from the high level transport activity of the D120C mutant, the conformational change should not be large enough to destroy the structure of the transporter. The Y357C mutant also showed a similar discrepancy. The mutant retained about 10% of the transport activity (Fig. 3), indicating that it also retains the structure of the transporter, whereas the conformational damage in Y357C may be larger than that in the D120C mutant. So, an additional mutant, D365C, was constructed as to the same predicted loop.

**Reactivity of Cysteine Residues Introduced into the N-terminal Half of Tn10-TetA with [14C]NEM and Its Prevention by AMS—**

*The binding of [14C]NEM to the cysteine residues introduced into the N-terminal half of Tn10-TetA was examined. As shown in Fig. 4, the reactivity of the S4C, S36C, S65C, L97C, and D120C mutants to [14C]NEM was high in the absence of AMS, indicating that these positions are located in regions exposed to the aqueous phase. On the other hand, the reactivity...*
Membrane Topology of the Tetracycline/H$^+$ Antiporter

The presence or absence of AMS, followed by labeling with $[^{14}C]$NEM as described in Fig. 1. The radiolabeled TetA proteins were visualized with a BAS-1000 Bio-Imaging Analyzer after immunoprecipitation and SDS gel electrophoresis. + and − indicate the presence and absence of AMS during the preincubation, respectively.

Table III

| Mutants | The amount of $[^{14}C]$NEM binding to the cysteine residues introduced into predicted loop regions of Tn10-TetA |
|---------|---------------------------------------------------------------------------------------------------------------|
| None    | mol bound NEM/100 mol of TetA                                                                                   |
| + AMS   |                                                                                                               |
| S4C     | 22.7                                                                                                           |
| S36C    | 27.5                                                                                                           |
| S65C    | 25.8                                                                                                           |
| L97C    | 27.8                                                                                                           |
| D120C   | 27.4                                                                                                           |
| S156C   | 15.5                                                                                                           |
| S199C   | 46.5                                                                                                           |
| S296C   | 9.4                                                                                                            |
| R238C   | 34.3                                                                                                           |
| T270C   | 43.2                                                                                                           |
| S296C   | 45.8                                                                                                           |
| S328C   | 51.9                                                                                                           |
| Y357C   | 16.6                                                                                                           |
| S360C   | 1.8                                                                                                            |
| D365C   | 44.4                                                                                                           |

Intact cells were incubated with or without 5 mM AMS for 30 min, followed by incubation with 0.5 mM $[^{14}C]$NEM for 5 min. The $[^{14}C]$NEM bound TetA was detected by SDS-gel electrophoresis of the immunoprecipitated TetA proteins as described under "Experimental Procedures." The amount of $[^{14}C]$NEM was calculated from the intensity of the Coomassie Brilliant Blue-stained bands.

The reactivity of $[^{14}C]$NEM to four of these six mutants was high (Fig. 5), confirming that these positions are located in loops exposed to the aqueous phase. However, the reactivity of $[^{14}C]$NEM to the S201C mutant was very low, and $[^{14}C]$NEM did not bind to the S360C mutant, indicating that these positions are hardly exposed to the aqueous phase. There are two alternative possibilities: 1) predicted loop 6–7 and loop 11–12 containing positions 201 and 360, respectively, might not be exposed to the aqueous phase. If this is the case, the topology of Tn10-TetA should be greatly changed. 2) Positions 201 and 360 may be unique points in the water-exposed loops at which the side chains are oriented into the protein interior. Our previous work on cysteine-scanning mutants as to loop 2–3 (29) indicated the presence of such a unique cryptic point in this water-exposed loop. In order to determine whether the segments containing these positions are exposed or not, we introduced cysteine residues into several positions close to positions 201 and 360. The S199C and D365C mutants showed high reactivity to $[^{14}C]$NEM in the absence of AMS (Fig. 6 and Table III). The Y357C mutant showed low but detectable reactivity to NEM (Fig. 6 and Table III). When the residues are located in the transmembrane region in TetA proteins, Cys mutants of these residues show no or very low binding (less than 0.1 mol of bound $[^{14}C]$NEM per mol of TetA) (30). Thus, it was confirmed that loop 6–7 and loop 11–12 are exposed to the aqueous phase.

The $[^{14}C]$NEM binding to the R238C, S296C, Y357C, and D365C mutants was completely prevented by preincubation with excess AMS, whereas the binding to the S199C, T270C, and S296C mutants was not affected by AMS at all (Figs. 5 and Table III). Binding of $[^{14}C]$NEM to the S201C mutant was also observed after preincubation with AMS (Fig. 5 and Table III). These results clearly indicated that large central loop 6–7, loop 8–9, and loop 10–11 are located on the cytoplasmic side, whereas loops 7–8, loop 9–10, and loop 11–12 are located on the outside surface of the cytoplasmic membrane. It may be surprising that position 296 is highly exposed to the outside medium, as judged from the reactivity of the S296C mutant to $[^{14}C]$NEM (Fig. 5B), in spite of the low hydrophilicity of the segment around this position and the lack of direct biochemical evidence of the periplasmic location of this loop (1, 18).
Membrane Topology of the Tetracycline/H\(^+\) Antiporter

**Fig. 6.** \[^{14}C\]NEM binding to the S199C, Y357C, and D365C mutants of Tn10-TetA and the prevention by preincubation of intact cells with AMS. Intact cells expressing these mutants were preincubated in the presence or absence of AMS, followed by labeling with \[^{14}C\]NEM as described in Fig. 1. The radiolabeled TetA proteins were visualized with a BAS-1000 Bio-Imaging Analyzer after immunoprecipitation and SDS gel electrophoresis. (+) AMS and (−) AMS indicate the presence and absence of AMS during the preincubation, respectively.

**DISCUSSION**

The membrane topologies of Tn10-TetA and its homolog, pBR322-TetA, have been predicted to include 12-transmembrane segments, and the N and C termini are located on the cytoplasmic side, as judged from the hydrophathy profile (1, 16). However, the predicted 12-transmembrane structure is not obvious because there are no distinct hydrophilic segments between predicted transmembrane segment (TM) 3 and TM4, and TM9 and TM10 (1). Hydropathy analysis of Tn10-TetA indicated the presence of 10 hydrophobic clusters of different sizes (1). Neither the presence of water-extruding loops nor the periplasmic location of segments between TM3 and TM4 and TM9 and TM10 has been directly supported by any biochemical data (1, 18). The results presented in this article for the first time clearly confirmed the validity of the 12-transmembrane topology of the TetA protein.

We performed site-directed chemical labeling of cysteine mutants with sulfhydryl reagents to determine the membrane topology of Tn10-TetA. This method is applicable to Tn10-TetA because wild-type Tn10-TetA has only one cysteine residue at position 377, but maleimide derivatives are not reactive to this cysteine (20) due to its being buried in the hydrophobic interior of the membrane. The criteria used for determining the location of an introduced cysteine are as follows. 1) When cysteine residues are not labeled with \[^{14}C\]NEM, they are embedded in the hydrophobic interior of the membrane or the folded loops, because the active species of a sulfhydryl group for reaction with maleimide is the deprotonated form. 2) When the labeling of cysteine residues with \[^{14}C\]NEM is not blocked by membrane-impermeant AMS, the residues are located on the cytoplasmic surface. 3) When the labeling with \[^{14}C\]NEM is blocked by AMS, the residues are located on the periplasmic surface.

We constructed 15 site-directed mutants in which cysteine residues were introduced into predicted periplasmic loops. Except for the S201C and S360C mutants, all mutants were highly labeled by \[^{14}C\]NEM in the absence of AMS. Since cysteine residues introduced into positions near 201 and 360 also showed good reactivity to \[^{14}C\]NEM, positions 201 and 360 are likely to be local cryptic points in the water-extruding loop regions. The binding of \[^{14}C\]NEM to the cysteine residues introduced into the predicted periplasmic loops was completely blocked by preincubation of intact cells with a membrane-impermeant SH reagent, AMS, whereas the binding to the cysteine residues introduced into the N-terminal segment and the predicted cytoplasmic loops was not affected by AMS, clearly indicating that the former cysteine residues are located on the periplasmic surface and the latter ones on the cytoplasmic surface. These results obviously support the 12-transmembrane structure of Tn10-TetA. Since the intervals between introduced cysteine residues are enough for the protein to traverse the membrane once but shorter than required to traverse it more than twice, the model of Tn10-TetA composed of more than 13 transmembrane segments is impossible.

The predicted periplasmic loops tend to be shorter than the cytoplasmic loops (Fig. 2). It has been suspected that TetA proteins are hardly exposed to the periplasmic surface of the membrane because no proteases can gain access from the periplasmic side (1). However, as judged from our results as to \[^{14}C\]NEM binding to the cysteines introduced into the periplasmic loops, they were certainly exposed to the aqueous phase, similar to the loops located on the cytoplasmic surface. The inaccessibility for proteases from the periplasmic side may be due to the protein conformation.

**REFERENCES**

1. Eckert, B., and Beck, C. F. (1989) J. Biol. Chem. 264, 11663–11670
2. McMurry, L., Petrucci, R. E., Jr., and Levy, S. B. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 3974–3977
3. Kaneko, M., Yamaguchi, A., and Sawai, T. (1985) FEBS Lett. 193, 194–198
4. Yamaguchi, A., Udagawa, T., and Sawai, T. (1990) J. Biol. Chem. 265, 4809–4813
5. Hillen, W., and Schollmeier, K. (1983) Nucleic Acids Res. 11, 525–539
6. Nguyen, T. T., Postle, K., and Bertrand, K. P. (1983) Gene (Amst.) 25, 83–92
7. Yoshioka, H., Bogaki, M., Nakamura, S., Ubuykata, K., and Konno, M. (1990) J. Bacteriol. 172, 6842–6849
8. Neufakh, A. A. (1992) Antimicrob. Agents Chemother. 36, 484–498
9. Henderson, P. J. F. (1990) J. Bioenerg. Biomembr. 22, 525–569
10. Yamaguchi, A., Iwasaki-Ohiba, Y., Ono, N., Kaneko-Ohdera, M., and Sawai, T. (1991) FEBS Lett. 282, 415–418
11. Yamaguchi, A., Akasaka, T., Ono, N., Someya, Y., Nakatanai, M., and Sawai, T. (1992) J. Biol. Chem. 267, 7490–7498
12. Yamaguchi, A., Nakatanai, M., and Sawai, T. (1992) Biochemistry 31, 8344–8349
13. Yamaguchi, A., Ono, N., Akasaka, T., Nouni, T., and Sawai, T. (1990) J. Biol. Chem. 265, 15525–15530
14. Seel, W., and Shatkin, A. J. (1992) Biochemistry 31, 3550–3554
15. Jessen-Marshall, A. E., Paul, N. J., and Brooker, R. J. (1995) J. Biol. Chem. 270, 16251–16257
16. Henderson, P. J. F., and Maiden, M. C. J. (1990) Philos. Trans. R. Soc. Lond. Biol. Sci. B326, 391–410
17. Yamaguchi, A., Adachi, K., and Sawai, T. (1990) FEBS Lett. 265, 17–19
18. Allard, J. D., and Bertrand, K. P. (1990) J. Biol. Chem. 265, 17699–17819
19. Luo, T. W., and Clarke, D. M. (1985) J. Biol. Chem. 270, 843–848
20. Yamaguchi, A., Kimura, T., Someya, Y., and Sawai, T. (1993) J. Biol. Chem. 268, 6496–6504
21. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 488–492
22. Taylor, J. W., Ott, J., and Eckstein, F. (1985) Nucleic Acids Res. 13, 8764–8785
23. Yamamoto, T., Tanaka, M., Nohara, C., Fukunaga, Y., and Yamagishi, S. (1981) J. Bacteriol. 145, 808–813
24. Someya, Y., Niwa, A., Sawai, T., and Yamaguchi, A. (1995) Biochemistry 34, 7–12
25. Stoker, N. G., Fairweather, N. F., and Spratt, B. G. (1982) Gene (Amst.) 18, 335–341
26. Moyer, H. S., Nguyen, T. T., and Bertrand, K. P. (1983) J. Bacteriol. 155, 549–556
27. Philipson, L., Anderson, P., Olshevsky, U., Weinberg, R., and Baltimore, D. (1978) Cell 13, 189–199
28. Yamaguchi, A., Kimura, T., and Sawai, T. (1994) J. Biochem. (Tokyo) 115, 958–964
29. Yamaguchi, A., Someya, Y., and Sawai, T. (1992) J. Biol. Chem. 267, 19155–19162
30. Kimura, T., Suzuki, M., Sawai, T., and Yamaguchi, A. (1996) Biochemistry, in press
