Charged Amino Acids at the Carboxyl-Terminal Portions Determine the Intracellular Locations of Two Isoforms of Cytochrome b₅*

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Outer mitochondrial membrane cytochrome b₅ (OMb), which is an isomorph of cytochrome b₅ (cyt b₅) in the endoplasmic reticulum, is a typical tail-anchored protein of the outer mitochondrial membrane. We cloned cDNA containing the complete amino acid sequence of OMb and found that the protein has no typical structural feature common to the mitochondrial targeting signal at the amino terminus. To identify the region responsible for the mitochondrial targeting of OMb, various mutated proteins were expressed in cultured mammalian cells, and the subcellular localization of the expressed proteins was analyzed. The deletion of more than 11 amino acid residues from the carboxyl-terminal end of OMb abolished the targeting of the protein to the mitochondria. When the carboxyl-terminal 10 amino acids of OMb were fused to the cyt b₅ that was previously deleted in the corresponding 10 residues, the fused protein localized in the mitochondria, thereby indicating that the carboxyl-terminal 10 amino acid residues of OMb have sufficient information to transport OMb to the mitochondria. The replacement of either of the two positively charged residues within the carboxyl-terminal 10 amino acids by alanine resulted in the transport of the mutant proteins to the endoplasmic reticulum. The mutant cyt b₅, in which the acidic amino acid in its carboxyl-terminal end was replaced by basic amino acid, could be transported to the mitochondria. It would thus seem that charged amino acids in the carboxyl-terminal portion of these proteins determine their locations in the cell.

The mitochondrion is bounded by a pair of highly specialized membranes, the outer and inner mitochondrial membranes, that play a crucial part in related activities. Each of the membranes contains a unique set of proteins, most of which are encoded in nuclear DNA, synthesized in the cytoplasm, and transported to the mitochondria. As expected from the “symbiotic hypothesis” of mitochondria, the outer membrane has similarities to the ER and/or plasma membranes that may have surrounded symbiotic bacteria (1, 2). The same or similar proteins, including cytochrome b₅ (cyt b₅; Refs. 3-5), NADH-cyt-b₅ reductase (6, 7), aldehyde dehydrogenase (8), glutathione S-transferase (9, 10), and the proto-oncogene product Bcl-2 (11, 12), are present in both membranes. To elucidate the mechanisms of the protein transport involving the development of the outer mitochondrial membrane, structural differences in targeting signals that direct proteins to each membrane system have to be defined.

There are two known isoforms of cyt b₅-like hemoprotein in a single cell: (a) cyt b₅ in the ER, and (b) outer mitochondrial membrane cyt b₅ (OMb; Refs. 4 and 5). Both are composed of three domains: (a) the amino-terminal hydrophilic domain, (b) the medial hydrophobic domain, and (c) the carboxyl-terminal hydrophilic domain. The amino-terminal domain has about 100 amino acid residues, contains a protoheme, extends out of the membrane, and participates in electron-transferring functions (4, 5, 13). Sequences of this domain of cyt b₅ and OMb are about 70% identical (14, 15). The hydrophobic domain consisting of about 20 amino acid residues is embedded in the lipid bilayer and functions for the insertion of proteins into the membranes as tail-anchored proteins (16). The carboxyl-terminal 10 amino acid residues of cyt b₅ are exposed to the luminal side of the ER cisterna (17, 18) and are required to target the cytochrome to the ER (19). Functions of the corresponding portion of OMb have remained unknown.

A long stretch of uncharged amino acid residues with the intervention of positively charged amino acids, which is a typical structural feature common to the mitochondrial targeting signal, was not found in the amino-terminal amino acid sequence obtained from the direct sequencing of the purified tryptic cytochrome and partial cDNA cloning (14, 15, 20). It has been reported that the carboxyl-terminal 43 amino acids of OMb contain sufficient information to target the cytochrome to the mitochondria (15). However, such a long stretch of the amino acid sequence, which is about one-third of the entire protein, may not be needed as the targeting signal.

In the present study, we obtained cDNA containing the complete amino acid sequence of OMb and examined which portion of the molecule has sufficient information for the mitochondrial targeting of OMb. Our evidence shows that the carboxyl-terminal 10 amino acid residues of OMb have sufficient targeting information, and that charged amino acids in this portion of cyt b₅ and OMb determine their locations in the cell.

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The abbreviations used are: ER, endoplasmic reticulum; cyt b₅, cytochrome b₅; OMb, outer mitochondrial membrane cyt b₅.
MATERIALS AND METHODS

Reagents and Biochemicals—Restriction and modifying enzymes were purchased from Takara (Kyoto, Japan), Nippon Gene (Toyama, Japan), and Toyobo (Shiga, Japan). The expression vector pSVL was from Pharmacia LKB. Dulbecco’s modified Eagle’s medium was obtained from Nissui, and fetal calf serum was obtained from Life Technologies, Inc. and Boehringer Mannheim. Peroxidase-conjugated and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG were from Cappel Products and EY Laboratory, respectively. The ECL Western blotting detection system was obtained from Amersham.

cDNA Cloning of Rat Liver OMb—A gt11 library constructed from poly(A)^+ RNA isolated from the liver of a male Harlan Sprague Dawley rat was screened for OMb using synthetic, mixed oligonucleotides designed from amino acid sequences of Glu-Glu-Thr-Trp-Met-Val (23-28) obtained from rat liver OMb (14). A cDNA clone with an insert of about 0.8 kilobase pair was obtained and subcloned into the pBluescript SK⁺ vector pBluescriptSKOMb.

Construction of the OMb Derivatives—All of the derivatives were inserted into pSVL for expression in mammalian COS-7 cells.

OMbΔN12; cDNA from which the amino-terminal 12 amino acids of OMb were deleted was obtained from pBluescriptSKOMb by digestion with BalI and EcoRl and ligated into pUC119 that had been previously digested with Spbl and EcoRI to create a new initiation codon. OMbΔC11, OMbΔC20, and OMbΔC31; the carboxyl-terminal deletion mutants were obtained from pBluescriptSKOMb by polymerase chain reaction using M13 sequencing primer M3 and oligonucleotides containing the appropriate premature termination codon as primers.

OMbB5C10 and OMbB5; cDNA fragments of OMb and cyt b₅ were inserted in tandem into M13mp18 to obtain M13mp18OMbB5. The deletion of the nucleotides coding the last 10 amino acids of OMb and the catalytic plus transmembrane domain of cyt b₅ and transmembrane plus the last 10 amino acids of OMb and catalytic domain of cyt b₅ was done to obtain OMbC10 and OMbB5, respectively, using a polymerase chain reaction and the appropriate oligonucleotides.

B5OMbC10; cDNA fragments of cyt b₅ and OMb were inserted in tandem into M13mp18 to obtain M13mp18B5OMb, and the deletion of the nucleotides coding the last 10 amino acids of cyt b₅ and the catalytic and transmembrane domain of OMb was done to obtain B5OMbC10 by using a polymerase chain reaction and the appropriate oligonucleotide.

Expression of Original and Mutated OMb in COS-7 Cells and Cell Fractionation—COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum in an atmosphere of 5% CO₂ at 37 °C. DNA transfection was carried out as described previously (19), using cationic liposomes (22). The cells were cultured for 17–48 h after the plasmid had been transfected into the cells.

Cells expressing original and mutated OMb were harvested in ice-cold STE buffer (0.25 M sucrose, 20 mM Tris-HCl, pH 8.0) containing 2 μg/ml leupeptin and 2 μg/ml pepstatin A, pH 8.0). After centrifugation of the suspension at 600 × g for 5 min, the pellet was homogenized gently in ice-cold STE buffer using a Teflon homogenizer. The homogenate was centrifuged at 600 × g for 5 min to precipitate the nucleus and unbroken cells, and the resultant supernatants were recentrifuged to separate the membrane fraction from the soluble materials at 280,000 × g for 15 min at 4 °C in an RT15A3 rotor (Hitachi). For cell fractionation studies, the post-nuclear supernatant was successively centrifuged at 9,000 × g for 7 min and 20,000 × g for 5 min in a RT15A3 rotor (Hitachi) to obtain the mitochondrial and lysosomal fractions, respectively. The supernatant was recentrifuged to separate microsomal membranes from cytosolic materials at 280,000 × g for 20 min in a RT15A3 rotor (Hitachi). All procedures were done at 4 °C.

Immunofluorescence Microscopy—Immunofluorescence microscopy was carried out as described previously (19). Four μg of plasmid DNA were transfected into COS-7 cells on a coverslip in a 3.5-cm dish. After incubation for about 12 h, the cells on the coverslips were fixed with 2% paraformaldehyde-0.1% glutaraldehyde in phosphate-buffered saline (10 mM phosphate buffer, pH 7.2, and 0.15 mM NaCl) for 15 min. The fixed cells were then treated with 1% Triton X-100 for 2 min for the purpose of permeabilization and were then incubated with rabbit anti-OMb or anti-cyt b₅ antibody and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG in phosphate-buffered saline containing 10 mM glycerol and 0.1% bovine serum albumin.

Analytical Procedures—The amount of wild-type and mutated proteins expressed in the transfected cells was estimated using immuno blot analysis. The subcellular fractions were subjected to SDS-polyacrylamide gel electrophoresis, followed by the transfer of the proteins to a polyvinylidene difluoride filter. Rabbit antibodies against cyt b₅ and OMb and peroxidase-conjugated goat anti-rabbit IgG were used for the primary and secondary antibodies, respectively. Amounts of proteins were measured using Nikon scantounch and NIH-Image as a densitometer.

RESULTS

cDNA Cloning and the Deduced Amino Acid Sequence of Rat Liver OMb—A cDNA clone for OMb of 845 nucleotides was isolated from a rat liver cDNA library in Agt11 (EMBL acces-
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The open reading frame starting from the putative ATG initiation codon codes for a peptide consisting of 146 amino acid residues, and the deduced amino acid sequence coincides with that obtained from direct amino acid sequencing of the purified tryptic cytochrome (14) and partial cDNA cloning (15), except for an additional 12 amino acid residues (Met-Ala-Thr-Pro-Glu-Ala-Ser-Gly-Ser-Gly-Arg-Asn) present at the amino-terminal end. The protein has no typical structural feature, i.e. a long stretch of uncharged amino acid residues with intervention of positively charged amino acids, in common with mitochondrial precursor proteins at the amino terminus, even in the newly determined 12 amino acid residues.

The Carboxyl-Terminal Hydrophilic Domain of OMb Is Sufficient for Transport to the Mitochondria—To determine the region responsible for targeting OMb to the outer mitochondrial membrane, various mutated proteins with a deletion in the amino- or carboxyl-terminal portion were constructed and expressed in cultured mammalian COS-7 cells, and the subcellular localization of the expressed proteins was analyzed (Fig. 1). OMbΔN12 has a deletion of the amino-terminal 12 amino acids and an additional proline residue just after the initiation methionine. OMbΔC11, OMbΔC20, and OMbΔC31 have deletions in the carboxyl-terminal 11, 20, and 31 amino acids, respectively (Fig. 1A). Intracellular localization of the original protein and four mutant proteins was observed using immunofluorescence microscopy. A string-like structure, which is a typical mitochondrial pattern of fluorescence, was observed in cells expressing the original cytochrome and OMbΔN12 (Fig. 1B). In contrast, cells expressing carboxyl-terminal deletion mutants (OMbΔC11, OMbΔC20, and OMbΔC31) were stained broadly over the cell, suggesting that these proteins localized in the cytoplasm; this was indeed confirmed by subcellular fractionation (Fig. 1C). Post-nuclear supernatant fractions from cells expressing the original protein and four deletion mutants were subjected to ultracentrifugation, and the distribution of these proteins between the cytoplasm and the particulate fraction, including the mitochondria, was analyzed by Western blotting. The original and OMbΔN12 proteins were recovered in the membrane fractions, whereas OMbΔC11 remained in the supernatant fraction. Thus, about 10 amino acid residues at the carboxyl-terminal end of OMb are required for the protein to target to the mitochondria.

To determine whether or not the carboxyl-terminal 10 amino acids of OMb contain sufficient information for mitochondrial targeting, the 10 amino acid residues of OMb were fused to the truncated cyt b₅ that had been deleted in the corresponding 10 amino acids, which was reported to be the ER-targeting signal (Ref. 19; Fig. 2). Cells expressing B5OMbC10 showed a typical mitochondrial fluorescence pattern, whereas OMbB5C10 was localized in the ER and plasma membrane, although the staining of the latter was faint. Thus, the last 10 amino acid residues of OMb do carry the information required for the protein to be targeted to the mitochondria, and the amino-terminal hydrophilic and transmembrane portions apparently have no targeting signal.

Mitochondrial Targeting of OMb Depends on the Positive Charge in the Carboxy-Terminal 10 Amino Acid Residues—Characteristic features of the mitochondrial targeting signals at the amino-terminal end of mitochondrial protein precursors were measured by immunoblotting. The distribution of monoamine oxidase protein (a) and NADPH-cytochrome c reductase activity (b) is also shown as markers for mitochondria and microsomes, respectively. The sum totals of all the values of the four fractions are given a value of 100%, and the various bars indicate the percentage of the total that each represents.

Fig. 2. Subcellular distribution of OMb-b₅ chimera proteins. A, construction of the chimerical proteins of OMb and cyt b₅. A and B, OMb and cyt b₅ portions, respectively. The middle portion of each construct represents transmembrane domain. B, indirect immunofluorescence microscopy of COS-7 cells expressing OMb-cyt b₅ chimera proteins. The original and chimerical proteins were expressed in COS-7 cells, and immunofluorescence stainings were carried out as described under "Materials and Methods." a, cells expressing OMbB5; b, cells expressing OMb5BC10; c, cells expressing B5OMbC10; and d, cells expressing cyt b₅. Anti-OMb (a and b) and anti-cyt b₅ (c and d) antibodies were used as the primary antibody for the staining of the cells. C, the subcellular distribution of deletion mutants. Cells expressing the original and mutant proteins of OMb and cyt b₅ were homogenized in STE buffer, and the mitochondrial (Mt), lysosomal (Lys), microsomal (Ms), and Cytosol (Cyt) fractions were fractionated as described under "Materials and Methods." The amounts of the expressed proteins (a) were measured by immunoblotting. The distribution of monoamine oxidase protein (b) and NADPH-cytochrome c reductase activity (c) is also shown as markers for mitochondria and microsomes, respectively. The sum totals of all the values of the four fractions are given a value of 100%, and the various bars indicate the percentage of the total that each represents.
are several positively charged amino acid residues with intervening short stretches of uncharged amino acids; the positively charged amino acids play a vital role in signaling functions (24). Two amino acids, Arg-137 and Lys-144, in the carboxy-terminal 10 amino acid residues of OMb are positively charged in the cell. To investigate their role in the targeting of OMb to the mitochondria, they were replaced with an alanine residue by site-directed mutagenesis (Fig. 3A). Cells expressing all mutant proteins, even a single substitution mutant, showed a reticular staining pattern that is characteristic of the ER in immunofluorescence microscopy (Fig. 3B). In cells expressing OMbK144A, both the mitochondria and ER were stained, whereas the ER was mainly stained in cells expressing OMbR137A and OMbRAKA. Essentially the same results were obtained in the subcellular fractionation studies (Fig. 3C); however, in cells expressing the cytochrome in both the ER and mitochondria, the ER contribution was more prominent in our subfractionation experiments than it was in studies done using fluorescence microscopy, probably because of differences in the surface areas of two organelles in the cell. Thus, both of the basic residues, especially Arg-137, are essential for the targeting function of the carboxy-terminal portion of OMb, and both are required for effective targeting. These observations mean that a single replacement of basic amino acids by a neutral one could alter the 10-amino acid sequence, which is a mitochondrial targeting signal, to an ER-targeting signal.

Conversion of the Signal for ER Targeting to Mitochondrial Targeting by the Substitution of a Single Charged Amino Acid—A comparison of amino acid sequences between the carboxy-terminal portions of OMb and cyt b5 revealed that the difference between them is the distribution of charged amino acid residues; OMb has a lysine at position 144, whereas cyt b5 has an aspartic acid at the carboxy-terminal end, although both have an arginine near the transmembrane portion and an acidic amino acid, Asp-142 for OMb and Glu-133 for cyt b5, at a position that is 5 amino acids down from this arginine (see Figs. 3A and 4A). To determine whether the ER-targeting signal of cyt b5 can be converted to a mitochondrial targeting signal, acidic amino acid residues of the carboxy terminus of cyt b5 were replaced by a neutral or basic amino acid (Fig. 4A). Cells expressing B5D134A and B5D134K showed a dual distribution pattern in the mitochondria and the ER, although in the latter cells, the ER pattern was faint (Fig. 4B). The subcellular fractionation study showed that a large amount of B5D134K protein was recovered in the mitochondrial fraction, although a considerable amount of the two mutant proteins remained in the ER (Fig. 4C). These observations mean that the introduction of a positively charged residue at the carboxyl terminus of cyt b5 changes the signal from an ER-targeting signal to a mitochondrial-targeting signal. When Arg-128, which is located just after the transmembrane domain of cyt b5, was replaced by a neutral amino acid, Gin, the reticular staining pattern was evident in cells expressing B5R128N, as it was in cells expressing OmBR137A (Figs. 3B and 4B). The residue at this position seems to have little role in the targeting function of the carboxyl-terminal portion of cyt b5.

**DISCUSSION**

We obtained evidence that OMb has an unprocessed mitochondrial targeting signal in its carboxy-terminal 10 amino acid residues, and that positively charged amino acids in this portion are essential for the signal. Although most mitochondrial proteins possess mitochondrial targeting signals in extension peptides at the amino-terminal ends of the precursor proteins (25), some proteins, including two outer mitochondrial membrane proteins, monoamine oxidase and Bcl-2, were found to have an unprocessed signal at the carboxyl-terminal portion (12, 26, 27). We reported earlier that the mitochondrial targeting signal of monoamine oxidase B is present within its carboxy-terminal 29 amino acid residues (26). Because this region has three positively charged amino acids and no negatively charged amino acids in a long stretch of uncharged residues, the positively charged residues seem to be essential for the signal function of this region; we did not determine the intracellular localization of the mutant proteins that replaced these basic amino acids for neutral or acidic ones. Thus, both OMb and monoamine oxidase have a type of targeting signal similar

**FIG. 3. Subcellular distribution of OMb derivatives with site-directed mutations at the carboxyl terminus.** A, construction of OMb derivatives with site-directed mutations at the carboxyl terminus. B, indirect immunofluorescence microscopy of COS-7 cells expressing mutant proteins. Mutated OMb proteins were expressed in COS-7 cells and subjected to immunofluorescence staining as described under "Materials and Methods." Anti-OMb antibody was used as the primary antibody to detect mutated proteins. a, cells expressing OmBR137A; b, cells expressing OmBKR144A; and c, cells expressing OmRRAKA. C, subcellular distribution of mutant proteins. Cell fractionation and the determination of mutant proteins were performed as described under "Materials and Methods." The subcellular fractions, the amount of mutant proteins and monoamine oxidase, and the NADPH-cytochrome c reductase activity are shown as described in the legend to Fig. 2.
Targeting Signal of Two Isoforms of Cyt b5

A. W. Steggles. Exon 4 is not included in the cDNA of cyt b5. The original and mutant proteins of cyt b5 were expressed in COS-7 cells and subjected to immunofluorescence staining as described under “Materials and Methods.” Anti-cyt b5 antibody was used as the primary antibody to detect mutated proteins. a, cells expressing B5R128N; b, cells expressing B5D134A; c, cells expressing B5D134K. c reductase activity is shown as described in the legend to Fig. 2.

We also found that charged amino acids at the carboxyl-terminal portions determine the intracellular locations of two isoforms of cyt b5. The replacement of positively charged amino acids in this portion of OMB with neutral ones resulted in the transport of the mutant protein to the ER; in contrast, the introduction of a positively charged residue into the carboxyl terminus of cyt b5 altered the intracellular location of this protein to the mitochondria instead of the ER. Thus, it seems apparent that the intracellular location of two isoforms of cyt b5 can be controlled by the charged amino acid at the carboxyl terminus.

The sorting of proteins to the mitochondria or the ER is not always strict. Bcl-2 was reported to be located in the ER and nuclear membranes as well as in mitochondria (11). The protein has two basic amino acids, His-Lys, located just after the transmembrane segment at the carboxyl-terminal end, and these residues could function as the targeting signal for mitochondrial transport. Such a function is probably insufficient for the signal, and some portion of the protein may leak out of the transport apparatus so that the protein is transported to or associated with the ER or other membranes. The same seems to hold true for mutants B5D134A and OMBK144A, which exhibited dual distribution to the mitochondria and the ER. The conversion of Asp-134 to Lys in cyt b5 did not produce a strong or adequate signal for mitochondrial transport, which was probably due to the interaction with carboxyl groups of Glu-133 and the carboxyl terminus, and not all of the protein was targeted to the mitochondria. Thus, targeting to the mitochondria and targeting to the ER seem to be competing pathways in the intact cell rather than mutually exclusive pathways.

Genes of human and bovine cyt b5 consist of six exons,2 and the introns and nucleotide sequences of the exon portion of the exon-intron junctions are almost the same as those for rat cyt b5. Furthermore, the nucleotide sequence of rat OMB cDNA is also similar to that of cyt b5, except for the section close to the junction between the third and fourth exons. The sequences of putative exon 1 (amino acid 1–54), exon 2 (amino acid 55–97), exons 3 plus 5 (amino acid 98–117), and exon 6 (amino acid 118–146) of rat OMB are 56, 71, 41, and 53% identical with those of rat cyt b5, respectively. Exons 1 and 2 consist of an amino-terminal heme-containing core and are involved in electron-transferring functions. Because exons 3 and 5 are hinge regions between the catalytic and membrane-anchoring domains, and exon 6 contains information on intracellular localization and membrane insertion, each exon has its own function. The nucleotide and amino acid sequences of exon 6 are shown in Fig. 5. The nucleotide sequence for the carboxyl-terminal 10 amino acid residues of cyt b5, which is the ER-targeting signal, is similar to that of the corresponding portion
Changing T at the stop codon of cyt b5 to A resulted in the introduction of the lysine residue to this portion of OMb, and this change, probably together with the replacement of Asp-134 with Ser, may direct this protein to the mitochondria. The acquisition of a positively charged residue at the carboxyl-terminal end may lead to the development of OMb from cyt b5, although the characteristic features of the ER targeting signal are less well understood.

REFERENCES
1. Margulis, L. (1971) Sci. Am. 225, 48–57
2. Uzzell, T. (1973) Science 180, 516–517
3. Fukushima, K., Ito, A., Omura, T., and Sato, R. (1972) J. Biochem. (Tokyo) 71, 447–461
4. Ito, A. (1980) J. Biochem. (Tokyo) 87, 63–71
5. Ito, A. (1980) J. Biochem. (Tokyo) 87, 73–80
6. Kuwahara, S., Okada, Y., and Omura T. (1978) J. Biochem. (Tokyo) 83, 1049–1059
7. Borgese, N., and Pietrini, G. (1986) Biochem. J. 239, 393–403
8. Nakayasu, H., Mihara, K., and Sato, R. (1978) Biochem. Biophys. Res. Commun. 83, 697–703
9. Morgenstern, B., Lundqvist, G., Andersson, G., Balk, L., and DePierre, J. W. (1984) Biochem. Pharmacol. 33, 3609–3614
10. Nishino, H., and Ito, A. (1990) Biochem. Int. 20, 1059–1066
11. Krajewski, S., Tanaka, S., Takayama, S., Schübler, M. J., Fenton, W., and Reed, J. C. (1993) Cancer Res. 53, 4701–4714
12. Nguyen, M., Millar, D. G., Yong, V. W., Korsmeyer, S. J., and Shore, G. C. (1993) J. Biol. Chem. 268, 25265–25268
13. D’Arrigo, A., Manera, E., Longhi, R., and Borgese, N. (1993) J. Biol. Chem. 268, 2802–2808
14. Lederer, F., Ghirri, R., Guiard, B., Cortial, S., and Ito, A. (1983) Eur. J. Biochem. 132, 95–102
15. De Silvestri, M., D’Arrigo, A., and Borgese, N. (1995) FEBS Lett. 370, 69–74
16. Kutay, U., Ahnert-Hilgen, G., Hartmann, E., Wiedemann, B., and Rapoport, T. A. (1995) EMBO J. 14, 217–223
17. Vergeres, G., Ramsden, J., and Waskell, L. (1995) J. Biol. Chem. 270, 3414–3422
18. Kuroda, R., Kinoshita, J., Hoshio, M., Mitoma, J., and Ito, A. (1996) J. Biochem. (Tokyo) 120, 828–833
19. Mitoma, J., and Ito, A. (1992) EMBO J. 11, 4197–4203
20. Osuka, J., and Heinemann, F. S. (1982) Biochim. Biophys. Acta 704, 163–173
21. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488–492
22. Ito, A., Miyazoe, R., Mitoma, J., Aka, T., Osaki, T., and Kunitake, T. (1990) Biochem. Int. 22, 235–241
23. Omura, T., and Takesue, S. (1970) J. Biochem. (Tokyo) 67, 249–257
24. Glick, B. S., Beasley, E. M., and Schatz, G. (1992) Trends Biochem. Sci. 17, 453–459
25. Hartl, F.-U., Pfanner, N., Nicholson, D. W., and Neupert, W. (1989) Biochim. Biophys. Acta 998, 1–45
26. Mitoma, J., and Ito, A. (1992) J. Biochem. (Tokyo) 111, 20–24
27. Zhu, W., Cowie, A., Wasyf, G. W., Penn, L. Z., Leber, B., and Andrews, D. W. (1996) EMBO J. 5, 4130–4141