Identification of Significant Residues for Homoallylic Substrate Binding of Micrococcus luteus B-P 26 Undecaprenyl Diphosphate Synthase*

Yugesh Kharel, Yuan-Wei Zhang, Masahiro Fujihashi‡, Kunio Miki‡§, and Tanetoshi Koyama¶

From the Institute of Multidisciplinary Research for Advanced Materials (formerly the Institute for Chemical Reaction Science), Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan, the Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan, and the RIKEN Harima Institute/SPring-8, Koto 1-1-1, Mikazukicho, Sayo-gun, Hyogo 679-5148, Japan

The primary structure of cis-prenyltransferase is totally different from those of trans-prenyltransferases (Shimizu, N., Koyama, T., and Ogura, K. (1998) J. Biol. Chem. 272, 19476–19481). To better understand the molecular mechanism of enzymatic cis-prenyl chain elongation, we selected seven charged residues in the conserved Region V and two of Phe-Ser motif in Region III of undecaprenyl diphosphate synthase of Micrococcus luteus B-P 26 for substitutions by site-directed mutagenesis and examined their effects on substrate binding and catalysis. Kinetic studies indicated that replacements of Arg-197 or Arg-203 with Ser, and Glu-216 with Gln resulted in 7-11-fold increases of $K_m$ values for isopentenyl diphosphate and 18–1200-fold decreases of $k_{cat}$ values compared with those of the wild-type enzyme. In addition, two mutants with respect to the Phe-Ser motif in Region III, F73A and S74A, showed 16–32-fold larger $K_m$ values for isopentenyl diphosphate and 12–16-fold lower $k_{cat}$ values than those of the wild-type. Furthermore, product analysis indicated that three mutants, F73A, S74A, and E216Q, yielded shorter chain prenyl phosphates as their main products. These facts together with the protein structural analysis recently carried out (Fujihashi, M., Zhang, Y.-W., Higuchi, Y., Li, X.-Y., Koyama, T., and Miki, K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4337–4342) indicated that the diphosphate moiety of homoaallylic substrate is electrostatically recognized by the three charged amino acids, Arg-197, Arg-203, and Glu-216, in Region V and the Phe-Ser motif in Region III, as well as catalytic function. It was suggested that the undecaprenyl diphosphate synthase takes a different mode for the binding of isopentenyl diphosphate from that of trans-prenyl chain elongating enzymes.

Prenyltransferases, also referred to as prenyl diphosphate synthases, are enzymes catalyzing the sequential condensation of isopentenyl diphosphate (IPP) with allylic diphosphates to produce linear prenyl diphosphates in the biosynthetic pathway of isoprenoid compounds, most of which are essential components of the cellular machinery, such as cholesterol, carotenoids, prenyl quinones, prenyl proteins, and dolichols. Although these condensation reactions are similar in terms of chemical mechanism, there are a number of enzymes having different specificities with respect to the chain length and double-bond stereochemistry of its final product. These enzymes can be classified into two major subgroups (cis and trans types) according to the geometry of the products and are extremely interesting from an enzymological viewpoint in which the reactions are regulated to proceed consecutively and terminate precisely at definite chain lengths depending on the specificities of individual enzymes (1, 2).

To date, the structural genes for many types of trans-prenyltransferases have been cloned and characterized. Multiple alignments of the deduced amino acid sequences of these trans-prenyltransferases show the presence of several conserved regions in the primary structures (3, 4). The conserved regions include two characteristic aspartate-rich motifs (DDXXD), which have been shown to be essential for the catalytic function as well as substrate binding by site-directed mutational analysis (5-8). Tarshis et al. (9) determined the crystal structure of chicken FPP synthase, in which most of the conserved regions are found in a large central cavity. Ohnuma et al. (10–14) and Tarshis et al. (15) have intensively investigated the product chain length determination mechanisms of short chain trans-prenyltransferases such as FPP synthase from Bacillus stearothermophilus or chicken and geranylgeranyl diphosphate (GGPP) synthase from Sulfolobus acidocaldarius.

On the other hand, cis-prenyltransferases catalyze cis-prenyl chain elongation to produce prenyl diphosphates with E,Z-mixed stereochemistry. In bacteria, sequential cis addition of IPP onto FPP as an allylic primer to give undecaprenyl diphosphate (UPP, C55) is catalyzed by UPP synthase. UPP is the direct precursor of glycosyl carrier lipid in the biosynthesis of bacterial cell wall polysaccharide components such as peptidoglycan and lipopolysaccharide. In eukaryotic cells, dehydrodolichyl diphosphate synthase responsible for cis-prenyl chain elongation catalyzes much longer chain elongation than does the bacterial enzyme. Dehydrodolichyl diphosphate is an essential precursor of carbohydrate carrier lipid in the biosynthesis of N-linked glycoprotein or glycosylphosphatidylinositol-anchored glycoprotein. Although cis-prenyltransferases have...
been partially purified and characterized from several organisms, no information has been available about the structure of cis-prenyltransferases until our recent isolation of the gene for UPP synthase of *M. luteus* B-P 26 by use of the colony autoradiography method (16). Surprisingly, the primary structure of the UPP synthase is completely different from those of trans-prenyltransferases. Homologous genes for the bacterial enzyme have been identified and isolated shortly afterward from *Escherichia coli*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* (17). Recently, the *Saccharomyces cerevisiae* RER2 gene and an *Arabidopsis thaliana* gene have been isolated and identified to encode dehydrodolichyl diphosphate synthase responsible for the biosynthesis of dolichol in eu- karyote (18, 19).

Comparison of the primary structures of several cis-prenyl chain elongating enzymes showed the presence of five conserved regions, but the DDXXD motifs typical of trans-prenyltransferases were completely absent (17). The crystal structure of *M. luteus* B-P 26 UPP synthase has recently been determined by us at 2.2 Å resolution as the first three-dimensional structure of cis-prenyl chain elongating enzymes (20). To our surprise, this enzyme shows a novel protein fold, which is totally different from the so called "isoprenoid synthase fold" that is thought to be a common structure for the enzymes relating to isoprenoid biosynthesis (21). This fact may raise the possibility that cis-prenyltransferases take quite a different mode for substrate binding and catalysis from that of trans-type enzymes. As the stereochemical course of prenyl chain elongation has been proved to be very similar between the trans- and cis-prenyltransferases as far as the chemistry on the substrates during the enzymatic reaction is concerned (22), it is of particular interest to learn the similarity and difference in the molecular mechanism of these enzymes.

To obtain information about the catalytic mechanism of enzymatic cis-prenyl chain elongation, seven of the conserved charged amino acid residues (Glu-193, Arg-197, Glu-201, Arg-203, Glu-216, Asp-221, and Asp-226) in Region V and two conserved residues (Phe-Ser motif, Phe-73 and Ser-74) in Region II were selected for substitutions by site-directed mutagenesis. This paper describes eludcation of Phe-73, Ser-74, Arg-197, Arg-203, and Glu-216, which participate in the binding of homooligyl substrate and catalytic function during cis-prenyl chain elongation.

**EXPERIMENTAL PROCEDURES**

**Materials and General Procedures**—[1-14C]IPP (1.95 TBq/mol) was purchased from Amersham Pharmacia Biotech. Nonlabeled IPP, FPP, and (Z,E,E)-GGPP (Z-GGPP) were synthesized according to the procedure of Davison et al. (23). Restriction enzymes and other DNA-modifying enzymes were from Takara Shuzo Co., Ltd. and Toyobo Co., Ltd. Potato acid phosphatase was a product of Sigma. *E. coli* B strain BL21 (DE3) was used as the host for expression of the target gene. Restriction enzyme digestions, transformations, and other standard molecular biology techniques were carried out as described by Sambrook et al. (24). Bacteria were cultured in Luria-Bertani (LB) or M9YG medium (3). All other chemicals were of analytical grade.

**Protein Sequence Alignment**—The protein sequence data base Swiss-Prot was searched to find amino acid sequences similar to that of *M. luteus* B-P 26 UPP synthase. Multialignment of amino acid sequences was performed using GENETYX genetic information processing software (Software Development).

**Construction of an Expression System for M. luteus B-P 26 UPP Synthase**—The *M. luteus* B-P 26 UPP synthase gene (16) was amplified for transformation of *E. coli* BL21 (DE3) and the overnight culture of *E. coli* cells carrying the pMluEX plasmid in LB medium containing 50 µg/ml ampicillin was inoculated into a 250-fold volume of M9YG medium containing 50 µg/ml ampicillin. The cells were grown at 37°C to an A600 value of 0.3, and isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM, and then the incubation was continued for an additional 3 h at 30°C. Overproduction of the proteins was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The *E. coli* cells that overproduced a mutated enzyme were harvested and disrupted by sonication in TE buffer (10 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA). The protein fraction precipitated from the 100,000 × g supernatant by 30–60% saturation with ammonium sulfate was dissolved in TE buffer containing ammonium sulfate at 30% saturation and chromatographed on a phenyl-Sepharose HP column (Amersham Pharmacia Biotech) with elution of a decreasing linear gradient of ammonium sulfate from 30 to 0% saturation. The UPP synthase fractions were desalted on a HTrap desalting column (Amersham Pharmacia Biotech), applied to a Mono Q HR 5/5 column (Amersham Pharmacia Biotech), and eluted with a stepwise gradient of 0–0.5 M NaCl in TE buffer. The fractions of the mutated enzyme were analyzed for purity by SDS-PAGE with Coomassie Brilliant Blue staining, and the fractions that showed more than 90% purity were used for further characterization. Protein concentrations were measured by the method of Bradford (26) with bovine serum albumin as a standard.

**IPP Synthase Assay and Product Analysis**—The enzyme activity was measured by determination of the amount of [1-14C]IPP incorporated into the respective substrate. The incubation mixture contained, in a final volume of 0.2 ml, 100 mM Tris-HCl buffer, pH 7.3, 0.5 mM MgCl₂, 15 µM FPP or Z-GGPP, 15 µM [1-14C]IPP (37 MBq/ml), 0.05% (w/v) Triton X-100, and a suitable amount of enzyme solution. After incubation at 37°C for 30 min, the reaction products were extracted with 1-butanol saturated with water, and the radioactive content in the butanol extract was measured with an Aloka LSC-1000 liquid scintillation counter.

For kinetic studies the concentration of allylic substrate FPP/Z-GGPP or homooligyl substrate [1-14C]IPP was varied, while the other substrate [1-14C]IPP or FPP/Z-GGPP was kept constant at 200 or 500 µM, respectively. Calculation of kinetic parameters was performed using EnzymeKinetics software, version 1.5 (Trinity Software).

To analyze the radioactive prenyl diphosphate products in the reaction mixture, the products were hydrolyzed to the corresponding alcohols with potato acid phosphatase according to our method reported previously (27). The alcohols were extracted with pentane and analyzed by reversed phase TLC plates (LKC-18, Whatman) with a solvent system of acetonewater (19:1). The positions of authentic standards were visualized with iodine vapor, and the distribution of radioactivity was detected by autoradiography. The TLC plates were exposed on a Fuji imaging plate at room temperature for 1 day and then analyzed with a Fuji BAS 1000 Mac bioimage analyzer. Relative amounts of products were calculated from the intensities of the photosstimulated luminescence on the imaging plate.

**RESULTS**

**Production of Site-directed Mutants of M. luteus B-P 26 UPP Synthase**—Fig. 1 shows the amino acid sequence alignment of Region V and the Phe-Ser motif in Region III of several cis-prenyltransferases. This alignment reveals that Region V contains seven highly conserved charged amino acids, including five negatively charged residues (three Glu residues at the...
positions of 193, 201, and 216 and two Asp residues at the positions of 221 and 226) and two positively charged Arg residues at positions 197 and 203 in the M. luteus UPP synthase. In Region III, two amino acid residues corresponding to positions 73 and 74 in the B. subtilis motif in Region III are indicated. Identical amino acid residues are reached a plateau at 240 nM Mg\(^{2+}\) to 600 nM. 

The residues mutated in this study are identified by the shaded characters in Region III, with Ser (R197S and R203S), respectively, so that the region corresponding to Phe-73 or Ser-74, F73A and S74A, showed 16–32-fold decreases in the enzymatic activity compared with Mg\(^{2+}\) ions for its catalytic function. The enzymatic activity is decreased rapidly above the concentration of 240 nM Mg\(^{2+}\). Furthermore, Mn\(^{2+}\), Ca\(^{2+}\), or Co\(^{2+}\) shows a weak stimulation on the enzymatic activity compared with Mg\(^{2+}\) or Zn\(^{2+}\). Elimination of the metal ions from reaction mixture by EDTA resulted in inactivation of the enzyme.

**Metal Ion Requirements**—Baba and Allen (28) have demonstrated that M. luteus UPP synthase requires divalent metal ions for its enzymatic activity. In our current analysis, Mg\(^{2+}\) is the most effective among the metal ions tested. The optimal concentration of Mg\(^{2+}\) is 240 nM when 60 nM enzyme was used, suggesting that one UPP synthase molecule utilizes four molecules of Mg\(^{2+}\) for its catalytic function. The enzymatic activity reached a plateau at 240 nM Mg\(^{2+}\) and stayed at that level up to 600 nM. Zn\(^{2+}\) shows an optimal concentration similar to Mg\(^{2+}\) for activation of the enzyme; however, the enzymatic activity decreased rapidly as the concentration of Zn\(^{2+}\) increased in excess of 240 nM. The other metal ions showed weak stimulation compared with Mg\(^{2+}\), Ca\(^{2+}\), or Co\(^{2+}\).

**Kinetic Analysis of the UPP Synthase Mutants**—Kinetic constants of these mutated enzymes as well as the wild-type were determined by employing [\(^{1-14}\)C]IPP and FPP or Z-GGPP as substrates, and the results are listed in Table I. All of the nine mutated enzymes showed comparable Michaelis constants (K\(_m\)) for allylic substrate FPP or Z-GGPP with those of the wild-type UPP synthase. In addition, the four mutants, E193Q, E201Q, D221A, and D226A, showed Km values for homoallylic substrate IPP within moderate folds to that of the wild-type and slightly decreased enzymatic activities. However, the substitution of positively charged residue Arg-197 or Arg-203 with Ser resulted in 7–11-fold larger k\(_{cat}\) values for IPP and 18–1200-fold lower k\(_{cat}\) values than those of the wild-type.

**Product Analysis**—One microgram each of the purified mutated enzyme as well as the wild-type was used for enzymatic reaction to analyze its reaction products. After enzymatic reaction, the reaction products were analyzed by thin-layer chromatography and by high-performance liquid chromatography.
action at 37 °C for 6 h using [1-14C]IPP and FPP as substrates, the radioactive prenyl diphosphate products were hydrolyzed to the corresponding alcohols. TLC analyses of the alcohols are shown in Fig. 2. Six mutants, including E193Q, R197S, E201Q, R203S, D221A, and D226A, showed product distribution patterns similar to that of the wild-type enzyme, which produced UPP (C55) as its major product as well as minor amounts of some intermediates having shorter prenyl chains and C50 product. Compared with the wild-type or other mutant enzymes, R197S and R203S have lower reaction rates in catalysis and product distributions of F73A, S74A, and E216Q mutants are significantly different from the wild-type. Replacement of charged amino acids in Region V and the two conserved residues in Region III is involved in the binding of allylic substrate.

Heat Stability of the UPP Synthase Mutants—Heat stabilities of the UPP synthase mutants were examined by analyzing their remaining activity after heat treatment at various temperatures for 30 min. All of the mutants exhibited thermostability profiles comparable with that of the wild-type UPP synthase (data not shown).

**DISCUSSION**

UPP, a product of UPP synthase, was known to be an essential precursor in bacterial cell wall construction. Disruption in normal expression of the enzyme is lethal for bacteria (29). Hence, a better understanding of the enzymology of cis-prenyl chain elongation will provide a practical strategy for designing new antimicrobial molecules.

The fact that all the mutant enzymes with respect to Regions III and V investigated in this work showed comparable $K_m$ values for allylic substrate FPP or Z-GGPP to those of the wild-type UPP synthase suggests that none of the seven charged amino acids in Region V and the two conserved residues in Region III is involved in the binding of allylic substrate. On the other hand, replacements of the positively charged residue Arg-197 or Arg-203 with Ser and the negatively charged residue Glu-216 with Gln resulted in a 18-fold decrease, while the substitution of Arg-197 or Arg-203 with Ser brought about a 1200-fold decrease to that of the wild-type. Thus, it is reasonable to assume that the three conserved amino acid residues, Arg-197, Arg-203, and Glu-216, participate in the binding of IPP as well as catalytic function. According to the results, we propose a hypothetical binding model for homoallylic substrate IPP to the UPP synthase as shown in Fig. 3. The diphenyl moiety of IPP binds directly with the positively charged guanidinium groups of Arg-197 and Arg-203 but binds with the carboxyl group of Glu-216, possibly through a Mg$^{2+}$ bridge. The two positively charged amino acids, Arg-197 and Arg-203, seem to be more important for catalytic function than Glu-216, because replacement of one of the Arg residues led to a heavy decrease in enzymatic activity. The two Arg residues with the Glu-216 residue from the other subunit, forming a charged triangle region, seem to be employed for IPP binding, because the Glu-216 in the same subunit with the two Arg residues resides in a remote position in the homodimeric structure of M. luteus B-P 26 UPP synthase, while the Glu-216 residue in the other subunit is located near to the two Arg residues (Fig. 3a).

The two mutants with respect to the conserved Phe-Ser motif in Region III, F73A and S74A, showed highly increased $K_m$ values for IPP with decreased $k_{cat}$ values. These results indicate that not only the binding affinity for IPP but also the catalytic function are affected by these amino acid replacements. This Phe-Ser (FS) motif resides in the position adjacent to the charged triangle formed by Arg-197, Arg-203, and Glu-216 (Fig. 3e). Thus, it is suggested that the highly conserved FS motif also plays an important role to contribute to the binding of IPP as well as catalytic function. Phe-73 seems to exert its hydrophobic nature on the hydrocarbon moiety of IPP, whereas Ser-74 may bind to the diphenyl group of IPP with hydrogen bonding. Replacement of Phe-73 with Ala may also alter the position of the hydroxyl group on Ser-74, which should be in a suitable proximity to the diphenyl moiety of IPP, resulting in a 16-fold decreased $k_{cat}$ value of F73A.

The UPP synthase reaction may be triggered by the elimination of the diphenyl ion of an allylic diphosphate to form...
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Fig. 3. A hypothetical model for the binding of IPP of M. luteus B-P 26 UPP synthase. a, stereoview of the hypothetical IPP binding model. White and blue envelopes show the molecular surfaces of two different subunits, respectively. Carbon, oxygen, and phosphorus atoms in substrate molecules are colored with white, magenta, and orange, respectively. The magnesium ion, which is located between the diphosphate group of IPP and carboxyl group of 216E, is shown as a brown sphere. This figure was prepared using programs MOLSCRIPT (32), GRASP (33), and Raster3D (34). b, schematic presentation of a plausible binding model of substrates.

an allylic cation at C1, which is attacked by the ε nitrogen atom at C4’ of IPP with the formation of a new C–C bond between C1 and C4’ as observed previously in FPP synthase reaction (30). The present binding scheme of IPP to the UPP synthase shows that the five residues, Phe-73, Ser-74, Arg-197, Arg-203, and 216, cooperatively bind the IPP molecule so as to hold the substrate molecule in the proper direction and to make C4’ of IPP to be close to C1 of IPP necessary for stereospecific condensation reaction (Fig. 3b).

Unlike the R197S and R203S mutants, which showed 10³ lower \( k_{\text{cat}} \) values but still produced UPP (C\(_{55}\)) as their main products, F73A, S74A, and E216Q showed significant differences in product chain length distribution from the wild-type enzyme. Replacement of Ser-74 with Ala or Glu-216 with Gln resulted in shortening the chain length of the major product, but the two mutants could produce a detectable amount of UPP (Fig. 2, lanes 3 and 8). However, a large amount of intermediate products (C\(_{20}\) and C\(_{30}\)) instead of UPP was produced by the F73A mutant (Fig. 2, lane 2). The Phe residue corresponding to position 73 in M. luteus B-P 26 UPP synthase is completely conserved among bacterial UPP synthases as well as eukaryotic dehydrodolichyl diphosphate synthases (Fig. 1). However, the recently cloned short cis-prenyl chain elongating enzyme Rv1086, which catalyzes the formation of E,Z-FPP, from Mycobacterium tuberculosis has a Leu residue at the corresponding position (31).

Among trans-prenyltransferases the aromatic amino acid residue that is located at the fifth position upstream to the first DDXD motif in chicken/B. stearothermophilus FPP synthase forms the floor of the putative allylic substrate binding pocket and is essential for controlling product chain length in trans-prenyl chain elongation (10, 15). The conserved Phe-73 in M. luteus B-P 26 UPP synthase seems to have a completely different function from that of the aromatic amino acid in trans-prenyltransferases. It is noteworthy that the three mutants, F73A, S74A, and E216Q, showed markedly increased \( K_{\text{m}} \) values for IPP. The \( K_{\text{m}} \) value for IPP of E216Q or S74A increased by 11- or 16-fold, while the substitution of Phe-73 with Ala brought about a 32-fold increase compared with the wild-type. The reason that these substitutions at Phe-73, Ser-74, or Glu-216 in the M. luteus B-P 26 UPP synthase led to shortening product chain length may be explained if the replacement results in the severe decrease of IPP binding, which may bring about relatively slow late IPP condensation to accumulate shorter prenyl chain intermediates.

It is noticeable that the UPP synthase takes a different mode for the binding of homoallylic substrate IPP from that of trans-prenyl chain elongating enzymes. It has been suggested that cis-prenyl chain elongating enzymes have evolved from a different ancestor from that of trans-prenyltransferases (16). To accelerate the understanding of the molecular mechanism of enzymatic cis-prenyl chain elongation, further studies on the structure-function relationship of cis-prenyl chain elongating enzyme are in progress involving site-directed mutagenesis as well as x-ray crystallographic analysis of the M. luteus B-P 26 UPP synthase with substrates bound.

REFERENCES
1. Ogura, K., Koyama, T., and Sagami, H. (1997) in Subcellular Biochemistry (Bittman, R., ed) Vol. 28, pp. 57–88, Plenum Press, New York.
2. Ogura, K., and Taya, T. (1989) Chem. Res. 98, 1263–1276.
3. Koyama, T., Ohata, S., Osabe, M., Takeshita, A., Yokoyama, K., Uchida, M., Nishino, T., and Ogura, K. (1993) J. Biochem. (Tokyo) 113, 355–363.
4. Chen, A., Kwon, P. A., and Poullier, C. D. (1994) Protein Sci. 3, 600–607.
5. Joly, A., and Edwards, P. A. (1993) J. Biol. Chem. 268, 26983–26989.
6. Song, L., and Poullier, C. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3044–3048.
7. Marrero, P. F., Poullier, C. D., and Edwards, P. A. (1992) J. Biol. Chem. 267, 21873–21878.
8. Koyama, T., Tajima, M., Sano, H., Doi, T., Koike-Takeshita, A., Ohata, S., Nishino, T., and Ogura, K. (1996) Biochemistry 35, 9533–9538.
9. Tarshis, L. C., Yan, M., Poullier, C. D., and Sacchettini, J. C. (1994) Biochemistry 33, 10871–10879.
10. Wang, K., and Ogura, S. (1999) Trends Biochem. Sci. 24, 445–451.
11. Ohnuma, S., Nakazawa, T., Hemmi, H., Hallberg, A., Koyama, T., Ogura, K., and Nishino, T. (1996) Biochemistry 35, 10087–10095.
12. Ohnuma, S., Hirooka, K., Hemmi, H., Ishida, C., Ohito, C., and Nishino, T. (1996) J. Biol. Chem. 271, 18831–18837.
13. Ohnuma, S., Hirooka, K., Ohito, C., and Nishino, T. (1997) J. Biol. Chem. 272, 5192–5198.
14. Ohnuma, S., Hirooka, K., Tsuura, N., Yano, M., Ohito, C., Nakane, H., and Nishino, T. (1998) J. Biol. Chem. 273, 26705–26713.
15. Tarshis, L. C., Proteau, P. J., Kellogg, B. A., Sacchettini, J. C., and Poullier, C. D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15018–15023.
16. Shimizu, N., Koyama, T., and Ogura, K. (1998) J. Biol. Chem. 273, 19476–19481.
17. Apfel, C. M., Takacs, B., Fountoulakis, M., Stieger, M., and Keck, W. (1999) Science 283, 483–492.
18. Sato, M., Sato, K., Nishikawa, S., Hirata, A., Kato, J., and Nakano, A. (1999) Mol. Cell. Biol. 19, 71–83.
19. Oh, S. K., Han, K. H., Ryu, S. B., and Kang, H. (2000) J. Biol. Chem. 275, 18482–18482.
20. Fujishashi, M., Zhang, Y.-W., Higuchi, Y., Li, X.-Y., Koyama, T., and Miki, K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4337–4342.
21. Sacchettini, J. C., and Poullier, C. D. (1997) Science 277, 1788–1789.
22. Koyama, T. (1999) Biosci. Biotechnol. Biochem. 63, 1671–1676.
23. Davison, V. J., Woodside, A. B., Neal, T. R., Strenler, K. E., Muehlbacher, M., and Poullier, C. D. (1986) J. Org. Chem. 51, 4768–4779.
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24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
26. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
27. Koyama, T., Fujii, H., and Ogura, K. (1985) Methods Enzymol. 110, 153–155
28. Baba, T., and Allen, C. M. (1980) Arch. Biochem. Biophys. 200, 474–484
29. Kato, J., Fujisaki, S., Nakajima, K., Nishimura, Y., Sato, M., and Nakano, A. (1999) J. Bacteriol. 181, 2733–2738
30. Poulter, C. D., and Billing, H. C. (1978) Acc. Chem. Res. 11, 307–311
31. Schulbach, M. C., Brennan, P. J., and Crick, D. C. (2000) J. Biol. Chem. 275, 22876–22881
32. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
33. Nicholls, A., Sharp, K. A., and Henig, B. (1991) Proteins 11, 281–296
34. Merritt, E. A., and Bacon, D. J. (1997) Methods Enzymol. 277, 505–524
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J. Biol. Chem. 2001, 276:28459-28464. doi: 10.1074/jbc.M102057200 originally published online May 9, 2001

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