Specific Binding of the TraY Protein to oriT and the Promoter Region for the \textit{traY} Gene of Plasmid R100* \\

Susumu Inamoto and Eiichi Ohtsubo \\
From the Institute of Applied Microbiology, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

The \textit{tra} gene product of plasmid R100 was purified as a hybrid protein, \textit{TraY-collagen}-\textit{\beta}-galactosidase. The hybrid protein as well as the \textit{TraY} protein, which was obtained by collagenolysis of the hybrid protein, specifically binds to an AT-rich 36-base pair sequence (here called \textit{sbY}A) within the region including the origin of transfer, \textit{oriT}. The \textit{oriT} region consists of highly conserved and nonconserved regions among R100-related plasmids, and \textit{sbY}A was located within the nonconserved region immediately adjacent to the conserved region. This supports the idea that the \textit{TraY} protein has a role as a component ofendonuclease in recognizing its own \textit{oriT} sequence. Unexpectedly, however, the hybrid protein and the \textit{TraY} protein were found to bind to two different AT-rich sequences (each 24 base pairs in length) in the promoter region preceding the \textit{tra} gene (here called \textit{sbY}B and \textit{sbY}C). This suggests that the \textit{TraY} protein may have another role in regulating the expression of its own gene. The "TAAA(\textit{A/T})TT" sequence motif observed in these binding sites might constitute a core sequence recognized by the \textit{TraY} protein. Mg\textsuperscript{2+} is not required for the specific binding of the \textit{TraY} protein.

Plasmid R100 confers conjugal transfer ability on its host, a process in which DNA is transmitted from one bacterial host to another. R100 and several other conjugative plasmids, such as F and R1, share homology in the \textit{tra} region which is responsible for DNA transfer (Sharp et al., 1973) and are considered to have the same conjugation system. The \textit{tra} region consists at least 26 genes which are organized into three main operons, \textit{traM}, \textit{traD}, and \textit{tra-Y-Z} (for recent reviews, see Ippen-Ihler and Minkley, 1986; Willetts and Skurray, 1987). Transcription of the \textit{tra-Y-Z} operon is positively regulated at the promoter \textit{Py}Z by the product of \textit{traD} (Gaffney et al., 1983; Fowler et al., 1983; Mullineaux and Willetts, 1985; Fowler and Thompson, 1986; Finlay et al., 1986a; Inamoto et al., 1988).

One of the initial events in DNA transfer upon expression of the \textit{tra} genes is the strand- and site-specific nicking at the origin of transfer, \textit{oriT}, by a plasmid-specific endonuclease (Everett and Willetts, 1980). It has been suggested that the \textit{TraY} protein is a component of the endonuclease and provides the DNA binding activity of the endonuclease to the \textit{oriT} region (Willetts and Maule, 1986). \textit{oriT} sequences of R100 and related plasmids (F, R1, ColB4, and P307) have been determined (Thompson et al., 1984; Ostermann et al., 1984; Finlay et al., 1986b; McIntyre and Dempsey, 1987; Goldner et al., 1987; Inamoto et al., 1988). The \textit{oriT} region contains a highly conserved region as well as a nonconserved region among these plasmids, although the minimal \textit{oriT} region is not clearly defined as yet. DNA nicking is considered to occur at several sites within the conserved region immediately adjacent to the nonconserved region (Thompson et al., 1984, 1989).

In this paper, we show that the \textit{tra} gene product specifically binds to the nonconserved region in \textit{oriT} as well as to the promoter region \textit{Py}Z preceding the \textit{tra} gene. We consider the possibility that the \textit{TraY} protein has a dual role: to recognize its own \textit{oriT} sequence as a component of a presumptive endonuclease responsible for nicking at \textit{oriT} and to regulate the expression of its own gene.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—The \textit{Escherichia coli} K12 strains used were JM109 (Yanisch-Perron et al., 1985) and MC1000 (Casadaban and Cohen, 1980).

Plasmids used were pUC19 (Yanisch-Perron et al., 1985), pSI200, pSI28, pSI87-XE1, pSI87-HE, pSI87-B19 (Inamoto et al., 1988), pSI87-ES1, pSI9, pSI11, pSI78, pYYS, and pYYS30-1. pSI87-ES1 was constructed by ligation, after pSI87-B19 was digested with EcoR\textit{v} and S\textit{al} and treated with DNA polymerase I (Klenow) in the presence of dNTPs. Plasmids (pSI9, pSI11, and pSI78) carried the EcoR\textit{v} fragments of R100 (r3, r5, and r6, respectively). They were constructed by cloning each fragment into the EcoR\textit{v} site of pUC19 in the same way as plasmid pSI28 carrying the EcoR\textit{v} fragment r4, as described previously by Inamoto et al. (1988). Plasmid pYYS5 (Yoshioka et al., 1987) and pYYO30-1 (Yoshioka et al., 1990) carried the S\textit{al}D fragment and G fragment of R100, respectively. The structures of several of these plasmids are shown in Fig. 2.

Media—Culture media used were LB broth, L-rich broth, and \textit{\phi}-medium (Yoshioka et al., 1987). \textit{\phi}-medium was used for transformation of plasmid DNA (Yoshioka et al., 1987). L-agar plates contained 1.5% Bacto-agar (Difco) in LB broth. Agar plates used to select ampicillin-resistant transformants contained 50 \textmu g/ml ampicillin (Sigma).

Reagents—dNTPs were purchased from Yamasa Co. \textit{r}DNA (type XX) and heparin were purchased from Sigma. BSA was purchased from Seikagaku Kogyo. \gamma\textsuperscript{[32P]}ATP (approximately 5000 Ci/mmol) was purchased from Amersham. SDS and DTT were purchased from Nakarai Chemicals, Ltd. Molecular mass standards for the ranges 200–45 kDa and 45–5 kDa were obtained from Bio-Rad Laboratories

* This research was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. 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.

† Recipient of a Fellowship of the Japan Society for the Promotion of Science for Japanese Junior Scientists.

The abbreviations used are: dNTP, deoxyribonucleoside 5'-triphosphate; bp, base pair(s); SDS, sodium dodecyl sulfate; L\textit{TT}, dithiothreitol; BSA, bovine serum albumin; ddCTP, 2',3'-dideoxy-cytidine 5'-triphosphate; ddGTP, 2',3'-dideoxyguanosine 5'-triphosphate; ddATP, 2',3'-dideoxyadenosine 5'-triphosphate; ddTTP, 2',3'-dideoxythymidine 5'-triphosphate; IHF, integration host factor; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; MES, N-(2-morpholino)ethanesulfonic acid.
and Bethesda Research Laboratories, respectively.

**Enzymes**—Restriction endonucleases (Accl, DraI, EcoRV, SacI, SfiI, Smal, SphI, and Stal), the Klenow fragment of DNA polymerase I, T4 DNA ligase, bacterial alkaline phosphatase, T4 polynucleotide kinase, and DNAase were obtained from Takara Shuzo Co. Restriction endonucleases, EcoRI, XhoII (Boehringer Mannheim Biochemicals), RsaI, Acal (New England Biolabs, Inc), and NsiI (Nippon Gene Co.) were used. Ribonuclease A was obtained from Sigma. They were used as recommended by their suppliers. 300 μg of collagenase (type VII) (Sigma) was dissolved in 340 μl of buffer B (10 mM Tris-HCl (pH 7.4), 200 mM NaCl, 10 mM CaCl₂, 10 mM 2-mercaptoethanol) (Inamoto et al., 1988) containing 50% (v/v) glycerol (here called buffer B-G10).

**DNA Preparation**—The E. coli strain harboring a plasmid was grown in L-rich broth. Plasmid DNA used for cloning, gel retardation assays, and DNAase I footprinting experiments was isolated from a large scale culture, as described by Ohtsubo et al. (1978). The crude lysis method (Machida et al., 1982) was used to prepare a small amount of plasmid DNA as large numbers of cell culture. An alkaline lysis method (Maniatis et al., 1982; Yoshiko et al., 1987) was used to prepare plasmid DNA for nucleotide sequencing, construction of recombinant plasmids, and for gel retardation assays.

**Preparation of the TraY Protein and Its Binding Specificity to the oriT Region**—The hybrid protein was purified according to the procedure described by Inamoto et al. (1988) with some modifications; the membrane fraction was removed by centrifugation at 100,000 × g for 1 h; a β-galactosidase-specific affinity column and columns PD-10 (Pharmacia LKB Biotechnology Inc.) were used at 6°C. The purified hybrid protein was dialyzed at 0°C against buffer B-G10, or one change of buffer B, containing 10% (v/v) glycerol (here called buffer B-G10). The hybrid protein in buffer B-G10 was frozen in dry ice and stored at −80°C, and the protein in buffer B-G50 was stored at −20°C.

The TraY protein was purified at 4°C as follows. The collagenase-lyzed hybrid protein (see below) in buffer B was replaced by CM buffer (10 mM K₂HPO₄, 10 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, 0.1% phenylmethylsulfonyl fluoride) containing 0.1% (w/v) glycerol and loaded on a CM-Sephadex C-55 (Pharmacia LKB Biotechnology Inc.) column equilibrated with CM buffer containing 0.1 M KCl. The column was washed with the same buffer, and the TraY protein was eluted with CM buffer containing 0.2 M KCl.

Protein concentration was determined using the method described by Bradford (1976) with BSA as standard.

**Collagenase of the TraY-Collegen-LacZ Fusion Protein**—Nineteen μl of the hybrid protein (790 μg of protein/ml) in buffer B was preincubated for 5 min at 28°C. Then, 0.5–1 μl of 10 mg/ml heparin was added, and the mixture was further incubated for 10 min. The samples were loaded onto a 4%–15% polyacrylamide sequencing gel containing 8 M urea. The bands were detected by staining with ethidium bromide. To examine sensitivity of the protein-DNA complex to SDS, SDS was added to a final concentration (10 μg/ml). To examine sensitivity of the protein-DNA complex to SDS, SDS was added to a final concentration (10 μg/ml).

**Restriction Assay**—Binding of the hybrid protein to plasmid DNA was carried out as follows: 0.5 μl of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) containing 400 μg/ml DNA (1 μg of pSI87-XE1 DNA or 200 μg/ml PUC19 DNA was mixed with 5.5 μl of distilled water, 2 μl of 5 X binding buffer A (100 mM Tris-HCl (pH 7.4), 250 mM NaCl, 50 mM MgCl₂, 12.5 mM EDTA, 25 mM DTT, 500 μg/ml BSA) and 2 μl of buffer B-G10 containing 10 μg/ml hybrid protein. The reaction mixture was incubated for 30 min at 28°C and then mixed with 2.5 μl of 5 X TAE buffer (200 mM Tris acetate (pH 8.0), 10 mM EDTA) containing 0.1% bromophenol blue and 50% (v/v) glycerol, immediately loaded onto a 0.7% agarose gel in 1 X TAE buffer, and electrophoresed at approximately 6 V/cm at room temperature. The bands were detected by staining with ethidium bromide. To examine sensitivity of the protein-DNA complex to heparin, heparin was added to a final concentration (10 μg/ml). To examine sensitivity of the protein-DNA complex to SDS, SDS was added to a final concentration (0.1%).

**Result**—Binding of the protein to the restriction fragments was carried out as follows. One μl of TE buffer containing 440 μg/ml DNA (XhoII-Rsal digest of pSI87-XE1 or 520 μg/ml DNA (Stal-Dral digest of pSI87-HE) were mixed with 4.5 μl of distilled water and 2 μl of 5 X binding buffer B (100 mM Tris-HCl (pH 7.4), 250 mM NaCl, 0.1 mM EDTA, 100 μg/ml BSA, 500 μg/ml BSA, 0.25% (v/v) glycerol) and preincubated for 5 min at 28°C. Two μl of the hybrid or TraY protein in buffer B-G10 was added and incubated for 20 min at 28°C. Then, 0.5 μl of 10 mg/ml heparin was added, and the mixture was further incubated for 10 min. The samples were loaded onto a 4%–15% polyacrylamide gel containing 8 M urea.

**RESULTS**

**TraY Protein and Its Binding Specificity to the oriT Region**—The TraY gene product was purified as a hybrid protein with collagen-β-galactosidase (LacZ) (Fig. 1A; Inamoto et al., 1988). The hybrid protein contains a 74-amino-acid sequence of the TraY gene product (75 amino acids) whose C-terminal Leu residue is substituted by the collagen-LacZ sequence (Inamoto et al., 1988). Digestion of this hybrid protein with...
collagenase resulted in production of two new proteins instead of the hybrid protein (Fig. 1B, lanes 1 and 2). Apparently, the larger one corresponds to the expected size of the LacZ portion (119 kilodaltons (kDa)), since it migrated at the same position as that of the native β-galactosidase (116.3 kDa) (Fig. 1B). The smaller one (9.3 kDa) was close to the expected molecular mass of the Tray portion (8.8 kDa), in which the C-terminal Leu residue in the native tray gene product was presumably displaced by Pro-Gly-Pro-Val. Although this protein (we call it Tray') could be purified to homogeneity using a cation exchange column (data not shown; see Materials and Methods), we used the mixture containing Tray' and LacZ portions generated upon gel electrophoresis for the studies to be described below on the properties of the Tray' protein.

To know whether or not the hybrid protein specifically binds to the oriT region, the plasmid pSI87-XEl DNA containing oriT or the vector plasmid pUC19 DNA (Fig. 2) was mixed with the hybrid protein and subjected to gel electrophoresis. Both samples migrated more slowly than the native plasmid DNAs in an agarose gel (data not shown), suggesting that a complex was formed between each plasmid DNA and the Tray protein. The specific binding of the Tray' protein to the 286-bp fragment (Fig. 2A, lane 3 or of some others in addition to the 286-bp fragment (Fig. 3A, lane 3) or of some others in addition to the 286-bp fragment (Fig. 3A, lane 3). Apparently, the presence of heparin, only the 286-bp XhoII-RsaI fragment containing the oriT region migrated slowly from its original position and formed a retarded band in a polyacrylamide gel (Fig. 3A, lanes 2 and 4), indicating that a complex was formed due to specific binding of the proteins to the 286-bp fragment. Addition of an excess of the proteins resulted in a mobility shift of all of the fragments (Fig. 3A, lane 3), indicating that the scale of the pUC19 sequence is different from those of the other sequences.

FIG. 1. Structure and analysis of the Tray-collagen-LacZ hybrid protein. A, structure of the hybrid protein. Numbers indicate positions of critical amino acid residues in the hybrid protein. Arrows indicate the expected collagenase cleavage sites. B, an SDS-polyacrylamide gel (11%), showing bands of proteins stained with Coomassie Brilliant Blue. Lane 1, purified hybrid protein (11 µg); lane 2, purified hybrid protein (11 µg) cleaved with collagenase (0.7 µg); lane 3, collagenase (0.9 µg); lanes 4 and 5, molecular mass standards. Positions of bands corresponding to the Tray-protein (Hybrid), β-galactosidase (LacZ), collagenase, and the Tray portion (Tray'), released upon collagenolysis of the hybrid protein, are indicated. Positions of molecular mass standards, myosin (200 kDa), β-galactosidase (116.3 kDa), phosphorylase B (92.5 kDa), lysozyme (14.3 kDa), and bovine trypsin inhibitor (6.2 kDa), are only shown.

FIG. 2. Structures of plasmids. A circular map of R100 is shown on top. Positions of the EcoRI fragments (r3, r4, r6, and r6), SalI-D fragment, and fragment G, which covers the region between r6 and SalI-D, are shown outside the circle. These fragments were inserted in pUC18 or pUC19 (see “Materials and Methods”). Plasmid pSI28 carrying the EcoRI fragment r4 is expanded together with the vector pUC19 sequence. Restriction fragments (A–E) generated upon digestion of pSI28 with AclI and EcoRI are shown. Ac, AclI site; Ec, EcoRI site. The two fragments C and D are also expanded to show locations of several tra genes (M, J, etc.), promoter region PyZ, and oriT. Structures of plasmids pSI87-XEl and pSI87-HE are shown under the expanded region. Relevant restriction sites on these plasmids for XhoII (X), RsaI (R), and AccI (Ac) are shown. The pUC19 sequence containing lacZ' (a thick open arrow) and po (the lacP0 region) is shown to indicate the orientation of the sequence within pSI28. Not shown. The scale of the pUC19 sequence is different from those of the other sequences.

mixed with the hybrid protein or the Tray' protein, and then subjected to gel electrophoresis. In the presence of heparin, only the 286-bp XhoII-RsaI fragment containing the oriT region migrated slowly from its original position and formed a retarded band in a polyacrylamide gel (Fig. 3A, lanes 2 and 4), indicating that a complex was formed due to specific binding of the proteins to the 286-bp fragment. Addition of an excess of the proteins resulted in a mobility shift of all of the fragments (Fig. 3A, lane 3), indicating that the scale of the pUC19 sequence is different from those of the other sequences.

To further prove this suggestion, pSI87-XEl DNA was digested with the restriction enzymes RsaI and XhoII (Fig. 2),...
recognized by the hybrid protein or the TraY protein within the 286-bp fragment, DNase I footprinting experiments were carried out. As shown in Fig. 4A, the hybrid protein as well as the TraY protein protect the region (nucleotide positions 27 to 60 shown in Fig. 5A) within the nonconserved region among plasmids related to R100. Protection against DNase I digestion by the hybrid and TraY proteins was also examined on the other strand of the fragment. Protection was observed for a region covering positions 28 to 61, as depicted in Fig. 5A. The protected sequence is very AT-rich (86% AT). Enhancement of cleavage by DNase I in the presence of the hybrid or TraY protein was not observed on either strand (see Fig. 4A).

TraY Protein Also Binds to Pzv, the Promoter Region Preceding tra—To see whether or not the hybrid protein or the TraY protein recognizes other parts of the tra region besides oriT, we carried out a gel retardation assay by two-dimensional gel electrophoresis. The DNA fragments generated by EcoRI and Acl double digestion of plasmid pSI28 containing the EcoRI fragment r4 (Fig. 2) were incubated with or without the hybrid protein and electrophoresed in the first dimension in an agarose gel (Fig. 6A). After treatment of the gel with SDS, electrophoresis was carried out in the second dimension. The fragments C and D from pSI28 (Fig. 2) formed several retarded bands seen as off-diagonal spots (see the brackets in Fig. 6B), indicating that these fragments are bound by the protein. Fragment C contains oriT, while fragment D contains Pzv, the promoter region preceding the traY gene (Fig. 2). Using the restriction fragments generated from plasmid pSI87-HE carrying the Pzv region (Fig. 2), the 173-bp DraI-Stul fragment, which was within the 322-bp region flanked by Acl and AccI sites (see Fig. 2), was specifically bound by the hybrid and TraY proteins. Restriction fragments generated from the plasmids, which carry EcoRI fragments (r3, r5, and r6) and Sall-D and G fragments containing different segments of tra of R100 (see Fig. 2), were not bound by the hybrid protein (data not shown).
proteins in the P\textsubscript{Y\textsubscript{2}} region, as shown in Fig. 5C. The sequences in ori\textsubscript{T} as well as in P\textsubscript{Y\textsubscript{2}} contain inverted repeat sequences (Fig. 5, A and C). The sequence in P\textsubscript{Y\textsubscript{2}} weakly bound by the hybrid or Tra\textsubscript{Y} protein shows poor homology with those in the other two binding sequences (Fig. 5C). These three binding sequences contain a "TAA(A/T)T" sequence motif, as shown in Fig. 5, A and B.

**DISCUSSION**

We have shown that the hybrid protein (Tra\textsubscript{Y}-collagen-\beta-galactosidase) and the Tra\textsubscript{Y} protein with a modified C-terminal end of the Tra\textsubscript{Y} protein bind to specific sites in the ori\textsubscript{T} region and in the promoter region (P\textsubscript{Y\textsubscript{2}}) preceding the tra\textsubscript{Y} gene. We have also shown that the hybrid and Tra\textsubscript{Y} proteins bind nonspecifically to DNA in the absence of heparin or even in the presence of heparin when an excess amount of the proteins is added. Such nonspecific binding is characteristic of essentially all DNA binding proteins. It has been reported that the replication initiator protein of plasmid R6K fused to collagen-\beta-galactosidase and the initiator moiety obtained by collagendalysis of the hybrid protein show the same specific DNA binding activity as the native initiator protein (Germino and Bastia, 1983, 1984). We believe therefore that the observed properties of the hybrid protein or the Tra\textsubscript{Y} protein in this paper represent those of the actual product of the tra\textsubscript{Y} gene. We thus refer below to both the hybrid protein and the Tra\textsubscript{Y} protein as the Tra\textsubscript{Y} protein.

As described under "Results," the Tra\textsubscript{Y} protein binds to a specific site in the plasmid-specific region immediately adjacent to the highly conserved region in ori\textsubscript{T} among plasmids related to R100. We named this site sby\textsubscript{A} (for specific binding site of the Tra\textsubscript{Y} protein) (Fig. 5A). This result indicates that
Fig. 7. Homologous sequences between the oriT and Pyz regions of R100-related plasmids. A, R; B, R1. Identical bases are indicated by asterisks. F has one homologous sequence which matches 10 out of 11 base pairs; R1 has one homologous sequence which shows 11 matches out of 12 base pairs. The coordinates of oriT of F are from Thompson et al. (1984), and those to Pyz of F are from Fowler et al. (1983). The coordinates to oriT of R1 are from Ostermann et al. (1984), and those of Pyz of R1 from Finlay et al. (1986a).

The TraY protein has a function in recognizing its own oriT sequence. Unexpectedly, the TraY protein also binds to two sites (we name these sbyB and sbyC, as represented in Fig. 5B) in the Pyz region. This suggests that the TraY protein may regulate the expression of its own gene.

sbyA consists of 30 base pairs, while both sbyB and sbyC consist of 24 base pairs. This difference in length may imply the difference in the number of protomers of the TraY protein involved in binding to each sby region. If so, the number of protomers bound to sbyA is 1.5 times as great as that bound to sbyB and sbyC. There are homologous sequences within the three regions recognized by the TraY protein (Fig. 5C). These sequences must play an important role in recognition by the TraY protein. These sequences contain the "TAA(T/T)TT" sequence motif (Fig. 5, A and B). This motif might constitute a core sequence recognized by the TraY protein.

Do the oriT and Pyz regions in other plasmids related to R100 contain the sequences which are recognized by their own TraY proteins? As shown in Fig. 7, there are homologous sequences between the oriT and Pyz regions in each of these plasmids. It is quite likely that these sequences in each plasmid are recognized by their own TraY protein. This is based on the consideration that tra genes of R100-related plasmids have the same organization and function.

The plasmid-specific endonuclease responsible for strand and site-specific nicking at oriT to initiate DNA transfer is thought to be a complex of the products of traY and traZ (Everett and Willetts, 1980). Recently, traZ activity was found to be dependent on the DNA sequence of traI encoding DNA helicase I (Traxler and Minkley, 1988). It is intriguing to speculate that the binding of TraY protein facilitates the ability of the TraI (or TraZ) protein to bind and nick at the highly conserved region. DNA helicase I activity of the TraI (or TraZ) protein unwinds the duplex DNA from a nick(s) to generate the single strand of the DNA that is transferred to the recipient.

The distance between the proposed nicking sites in oriT and sbyA is 59-74 base pairs (see Fig. 5A). Upon contact with the TraY protein, the TraI (or TraZ) protein may induce a change in DNA structure such as looping of the intervening region between the nicking sites and sbyA to interact with the region adjacent to or around the nick sites. It is known that transfer of R100 and F from the mutant lacking IHF (integration host factor) is reduced (Dempsey, 1987; Gamas et al., 1987) and that there is a possible IHF recognition site in the nonconserved region in oriT at nucleotide positions 9 to 21 in Fig. 5A (McIntire and Dempsey, 1987), adjacent to sbyA. It is interesting to consider that IHF may promote the interaction of the TraI (or TraZ) protein with the TraY protein and with the highly conserved region in oriT.
Specific binding of the TraY protein to oriT and the promoter region for the traY gene of plasmid R100.
S Inamoto and E Ohtsubo

*J. Biol. Chem.* 1990, 265:6461-6466.

Access the most updated version of this article at [http://www.jbc.org/content/265/11/6461](http://www.jbc.org/content/265/11/6461)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/265/11/6461.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/265/11/6461.full.html#ref-list-1](http://www.jbc.org/content/265/11/6461.full.html#ref-list-1)