Translational Repression by Bacteriophage MS2 Coat Protein Expressed from a Plasmid
A SYSTEM FOR GENETIC ANALYSIS OF A PROTEIN-RNA INTERACTION*

David S. Peabody
From the Departments of Cell Biology and Biochemistry, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

The coat protein of bacteriophage MS2 is a translational repressor. It inhibits the synthesis of the viral replicase by binding a specific RNA structure that contains the replicase translation initiation region. In order to begin a genetic dissection of the repressor activity of coat protein, a two-plasmid system has been constructed that expresses coat protein and a replicase-β-galactosidase fusion protein from different, compatible plasmids containing different antibiotic-resistant determinants. The coat protein expressed from the first plasmid (pCT1) represses synthesis of a replicase-β-galactosidase fusion protein encoded on the other plasmid (pRZ5). Mutations in the translational operator or in coat protein result in constitutive synthesis of the enzyme. This permits the straightforward isolation of mutations in the coat sequence that affect repressor function. Because of the potential importance of cysteine residues for RNA binding, mutations were constructed that substitute serines for the cysteine residues for RNA binding. Mutations were introduced that affect the repressor function of coat protein.

At late stages of infection of Escherichia coli by bacteriophage MS2, the viral coat protein binds a stem-loop structure in the viral RNA which contains the ribosome binding site of the replicase gene, thus repressing replicase synthesis (1). The translational operator consists of a 21-nucleotide stem-loop whose specific three-dimensional structure forms the coat protein-binding site (2,3). This small piece of RNA may also serve as the signal for encapsidation of the RNA genome.

Although the basic rules for the structure of the MS2 translational operator have been investigated, we know very little about which aspects of coat protein structure account for its repressor activity. Apart from the putative interaction between a uridine residue of the operator and a cysteine residue of coat protein (4) no likely sites of RNA contact with the repressor have been identified. I have begun a dissection of the translational repressor activity of MS2 coat protein by constructing a genetic system that places synthesis of a hybrid replicase-β-galactosidase enzyme under translational control of coat protein. This permits the straightforward isolation of mutations that affect the repressor function of coat protein.

MATERIALS AND METHODS

Plasmid Constructions—The manipulation of MS2 sequences has been facilitated by the existence of a cloned cDNA of the viral genome (5) and by the availability of the nucleotide sequence (6-8). To construct the plasmid called pCT1 (Fig. 1) the coat protein-coding sequence was excised from the cDNA by cleavage at a XbaI site a short distance upstream of the coat initiation codon, and at a HaeIII site situated just downstream of the coat termination codon. The fragment containing the coat sequence was isolated by agarose gel electrophoresis and inserted by ligation into pUC19 (9) between the XbaI and Smal sites. This places the coat sequence under transcriptional control of the lac promoter. The plasmid pUC19 is a ColEl-type plasmid that confers ampicillin resistance to transformed bacteria.

Mutations were introduced into the coat gene by oligonucleotide-directed site-specific mutagenesis according to the method of Kunkel et al. (10). To provide a single-stranded template for mutagenesis, the coat sequence was excised from pCT1 with HindIII and KpnI and inserted into pUC19 (9). To test the importance of cysteine residues for translational repressor activity, mutations were introduced that converted the codons for each of the 2 cysteine residues to serine codons. Specifically, the Cys-46 codon was converted from UGU to UCU and the Cys-101 codon was changed from UGC to AGC. After confirmation of their structures by DNA sequence analysis, the mutant sequences were reintroduced into pUC19. The resulting plasmids are called pCTC46S and pCTC101S.

The plasmid pUCter3, which was used as a control in some experiments, was constructed by insertion of a synthetic 21-base pair fragment, containing termination codons in all three reading frames, between the Smal and EcoRI sites of pUC19. It possesses the properties of pCT1 except the ability to express coat protein.

The plasmids pRZ5 and pRZ6 were designed to create replicase-lacZ fusions which would be transcribed from the lac promoter on plasmids from a different incompatibility group than the pCT1 plasmid (ColEl-type) described above. Their construction was performed in two steps beginning with a plasmid called pNS3 (11), a derivative of pBR322 which contains most of the lac operon (lacZYA) under control of the PL promoter. The lacZ sequence of pNS3 lacks a translation initiation site. It has been replaced with a synthetic sequence containing recognition sites for EcoRI, Smal, and BamHI. This made it possible to create replicase-lacZ fusions whose translation would be entirely dependent on the replicase translation initiation sequence. The plasmids called pNSRZ5 and pNSRZ6 resulted from the insertion of synthetic DNA fragments bearing the wild-type (pNSKZ5) or a mutant (pNSRZ6) translational operator between the EcoRI and BamHI sites of pNS3. The sequences of the synthetic oligonucleotides used in the construction of pNSRZ6 were 5'-AATT-
Translation Repression by Bacteriophage MS2 Coat Protein

CCAACATGAGGATTACCCATGTCGAAG-3' and its complement 5'-GATCCTTCGACATGGGTAATCCTCATGTTTG-3'. The synthetic oligonucleotides containing the operator mutant of pNSRZ6 were 5'-AATTCAAACATGAGGAAAACCCATGTCGAAG-3' and its complement 5'-GATCCTTCGACATGGGTAATCCTCATGTTTG-3'. The insertions of these sequences into pNSR35 results in the creation of replicase-lacZ fusions in which the first three codons of replicase were replaced by lac promoter codons. These plasmids are used with ColE1-type replicons and confer ampicillin resistance. I wanted to place the replicase-lacZ fusions on a plasmid compatible with ColE1-type replicons so they could be stably maintained in cells which also contain pCT1. For this reason the replicase-lacZ fusions were excised from the pNSRZ constructs and joined in a three-piece ligation fragment from pUC19, and to o Herculease containing the lac promoter. The plasmid pACYC184 (12) contains a P15A origin of replication and a chloramphenicol-resistance gene. This was necessary in order to introduce and maintain the pRZ plasmids in strains that also contain pCT1. The constructions are described in detail below. The lac promoter to drive transcription of the replicase-lacZ gene fusion in the pRZ plasmids was derived from a plasmid called pUCd1, a derivative of pUC19 from which the translation initiation region of lacZ has been deleted. The deletion was introduced by treatment of HindIII-digested pUC19 with Bal-31 nuclease, followed by ligation with T4 DNA ligase. The bidirectional deletion thus generated resulted in loss of the pUC19 polylinker sequences. The translation initiation region of lacZ has been deleted. The deletion was introduced by treatment of HindIII-digested pUC19 with Bal-31 nuclease, followed by ligation with T4 DNA ligase. The bidirectional deletion thus generated resulted in loss of the pUC19 polylinker sequences. The lac nromoter, the translational operator prevent translational repression. So, to create the replicase-lacZ fusions translating from the lacZ' initiation site into the region containing the lacZ' initiation site, the synthesis of a replicase-P-galactosidase fusion protein encoded by one plasmid is under translational control of coat protein encoded by a second plasmid. This permits one to take advantage of the genetic selection and screening schemes which were devised during the study of the lac operon (13). In order to stably maintain both types of recombinant in the same bacterial strain they were constructed from plasmids which belong to different incompatibility groups and confer resistance to different antibiotics. Thus, pCT1 and its mutant derivatives pCTC46S and pCTC101S were constructed because of the assertion that a cysteine is necessary for RNA binding activity (4, 16).

The pRZ derivatives were excised from pNSRZ5 and pNSRZ6 by digestion with EcoRI and SalI, and the vector fragment was derived from pACYC184 by digestion with ClaI and SalI. The pRZ derivatives formed by the ligation of these three fragments are shown in Fig. 1. These plasmids were introduced into a host strain which is an F' derivative of CSH41 (lacpro, gale, thi, see Ref. 13). Growth Medium and Assays—Growth media and methods for the assay of β-galactosidase were as described by Miller (13). Indicator plates contained LB medium and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) at a concentration of 40 μg/ml. The growth of recombinant strains was performed on minimal medium (M9) containing 0.2% glycerol and chloramphenicol. Plasmid pRZ5 contains a synthetic translational initiation region of the MS2 coat protein, which the synthesis of a replicase-lacZ fusion protein encoded by one plasmid is under translational control of coat protein encoded by a second plasmid. This permits one to take advantage of the genetic selection and screening schemes which were devised during the study of the lac operon (13). In order to stably maintain both types of recombinant in the same bacterial strain they were constructed from plasmids which belong to different incompatibility groups and confer resistance to different antibiotics. Thus, pCT1 and its mutant derivatives pCTC46S and pCTC101S were constructed because of the assertion that a cysteine is necessary for RNA binding activity (4, 16).

The pRZ5 and pRZ6 constructs produce a replicase-β-galactosidase fusion protein also under control of the lac promoter (Fig. 1). They contain a P15A replication origin (from pACYC184, Ref. 12) and render cells resistant to chloramphenicol. Plasmid pRZ5 contains a synthetic translational operator corresponding to the sequence of the wild-type operon of the MS2 replicase gene (1-3, 8). On the other hand, pRZ6 was constructed with a mutant sequence designed to take advantage of the antigenic selection and screening schemes which were devised during the study of the lac operon (13). In order to stably maintain both types of recombinant in the same bacterial strain they were constructed from plasmids which belong to different incompatibility groups and confer resistance to different antibiotics. Thus, pCT1 and its mutant derivatives pCTC46S and pCTC101S were constructed because of the assertion that a cysteine is necessary for RNA binding activity (4, 16).

The pRZ5 and pRZ6 constructs produce a replicase-β-galactosidase fusion protein also under control of the lac promoter (Fig. 1). They contain a P15A replication origin (from pACYC184, Ref. 12) and render cells resistant to chloramphenicol. Plasmid pRZ5 contains a synthetic translational operator corresponding to the sequence of the wild-type operon of the MS2 replicase gene (1-3, 8). On the other hand, pRZ6 was constructed with a mutant sequence designed to take advantage of the antigenic selection and screening schemes which were devised during the study of the lac operon (13). In order to stably maintain both types of recombinant in the same bacterial strain they were constructed from plasmids which belong to different incompatibility groups and confer resistance to different antibiotics. Thus, pCT1 and its mutant derivatives pCTC46S and pCTC101S were constructed because of the assertion that a cysteine is necessary for RNA binding activity (4, 16).

The pRZ5 and pRZ6 constructs produce a replicase-β-galactosidase fusion protein also under control of the lac promoter (Fig. 1). They contain a P15A replication origin (from pACYC184, Ref. 12) and render cells resistant to chloramphenicol. Plasmid pRZ5 contains a synthetic translational operator corresponding to the sequence of the wild-type operon of the MS2 replicase gene (1-3, 8). On the other hand, pRZ6 was constructed with a mutant sequence designed to take advantage of the antigenic selection and screening schemes which were devised during the study of the lac operon (13). In order to stably maintain both types of recombinant in the same bacterial strain they were constructed from plasmids which belong to different incompatibility groups and confer resistance to different antibiotics. Thus, pCT1 and its mutant derivatives pCTC46S and pCTC101S were constructed because of the assertion that a cysteine is necessary for RNA binding activity (4, 16).

The pRZ5 and pRZ6 constructs produce a replicase-β-galactosidase fusion protein also under control of the lac promoter (Fig. 1). They contain a P15A replication origin (from pACYC184, Ref. 12) and render cells resistant to chloramphenicol. Plasmid pRZ5 contains a synthetic translational operator corresponding to the sequence of the wild-type operon of the MS2 replicase gene (1-3, 8). On the other hand, pRZ6 was constructed with a mutant sequence designed to take advantage of the antigenic selection and screening schemes which were devised during the study of the lac operon (13). In order to stably maintain both types of recombinant in the same bacterial strain they were constructed from plasmids which belong to different incompatibility groups and confer resistance to different antibiotics. Thus, pCT1 and its mutant derivatives pCTC46S and pCTC101S were constructed because of the assertion that a cysteine is necessary for RNA binding activity (4, 16).
Translation Repression by Bacteriophage MS2 Coat Protein

Production of β-Galactosidase Activity in Strains Bearing the Various Recombinant Plasmids—Escherichia coli strain CSH41 was transformed with pUCter3, pCT1, pCTC46S, or pCTC101S and pRZ5 or pRZ6. The resulting eight strains are listed in Table I. The ability of each of them to produce β-galactosidase was determined: 1) by solution assay using the o-nitrophenyl-β-D-galactoside substrate as described by Miller (13); 2) by the intensity of the blue color of colonies on medium containing X-gal; and 3) by growth on minimal medium containing P-gal. Table I shows the results of these experiments. By these criteria this two-plasmid system faithfully represents the translational repression observed in MS2-infected cells. The presence of pCT1, which produces wild-type coat protein, results in about a 30-50-fold repression of β-galactosidase synthesis from pRZ5. Failure to produce coat protein (e.g. pUCter3), or the production of a defective coat protein (e.g. pCTC46S) results in loss of repression of β-galactosidase synthesis. Mutation of the translational operator (as in pRZ6) also results in a constitutive phenotype. For reasons that are not understood, the mutations in pRZ6 also result in a 2-fold increase in expression of β-galactosidase in the absence of coat protein (pUCter3 samples in Table I). The operator variant present in pRZ6 permits only partial repression by wild-type coat protein.

Purification of Plasmid-produced Coat Protein—The ability of coat protein to be interconverted between forms of 2.5 × 10^6 daltons (capsids) and forms of dramatically lower molecular weight (monomer molecular weight is 1.4 × 10^4) provides a basis for the purification of plasmid-produced coat proteins. Sepharose CL-4B has previously been used in the purification of intact MS2 virus (18). The exclusion limit of this gel-filtration matrix is appropriate for the separation of the viral particle from species of higher and lower molecular weights. As a first step in the purification of coat protein from CSH41(pCT1), an extract of the cells was prepared as described under “Materials and Methods” and applied to a column of Sepharose CL-4B. Fractions were assayed for the presence of coat protein by SDS-polyacrylamide gel electrophoresis and Western blotting of fraction aliquots. The elution profile is shown in Fig. 2B. Electrophoretic analysis (Fig. 3) of the peak fractions from the Sepharose column indicates a substantial enrichment for coat protein. A second passage through the Sepharose column of pooled fractions containing coat protein resulted in an additional enrichment. Material from the peak fractions was pooled, concentrated by ammonium sulfate precipitation, dialyzed against Sepharose column buffer, and acetic acid was added to a final concentration of 50%. This acid-denatured material was applied to a 1.5 × 45-cm column of Sephadex G-75 in 10 mM acetic acid, 50 mM NaCl. Fraction aliquots were subjected to SDS-polyacrylamide gel electrophoresis and stained with Coomassie Blue. Contaminants were of higher molecular weights and eluted from the column before coat protein. The purity of coat protein thus isolated was determined by gel electrophoresis. Fig. 3 indicates the purity of coat protein at each stage of the purification procedure.
Translation Repression by Bacteriophage MS2 Coat Protein

FIG. 3. Stages of coat protein purification from CSH41 (pCT1) as monitored by SDS-polyacrylamide gel electrophoresis. 1, whole cell lysate of CSH41; 2, lysate of CSH41(pCT1); 3, pooled coat protein-containing fractions after a single passage over Sepharose CL-4B; 4, pooled coat protein-containing fractions after a second passage through the Sepharose column; 5, purified coat protein after chromatography on Sephadex G-75; 6, purified MS2 standard.

FRACTION No. pCTC46S

FRACTION No. pCTC101S

FRACTION No. pCT1

FRACTION No. pCTC46S

FRACTION No. pCTC101S

FRACTION No. pCT1

Analysis of the Plasmid-produced Coat Proteins—It was possible that the repressor-defective phenotype of C46S and the partially defective phenotype of C101S were the results of a failure of the proteins to adopt or maintain the native conformation. It has been previously demonstrated that mutations that prevent proper folding, or that result in decreased thermodynamic stability, often also result in rapid intracellular degradation and reduced steady state levels of the specific protein (for example, see Ref. 19). To determine the amounts of coat protein produced by each of the plasmid constructs, equal quantities of cell extract from each strain were applied to a 17.5% polyacrylamide gel containing SDS and fractionated by electrophoresis. After transfer to a nitrocellulose membrane, the coat proteins were visualized using rabbit anti-MS2 serum and 125I-protein A. Fig. 4 shows the results of this experiment. Strains containing pCT1 produced coat protein at levels corresponding to about 2 mg/liter of culture. The C46S mutant directs the synthesis of coat protein at levels that are reduced about 5-fold. By analogy to Pakula et al. (19) C46S may be defective for folding and/or stability.

To assess more directly the extent to which the mutant proteins adopt the native conformation, we subjected the mutant coat proteins to chromatography on a column of Sepharose CL-4B. We make the assumption that only properly folded coat proteins are capable of forming virus-like particles. This seems reasonable, since the formation of a capsid requires multiple intersubunit contacts. The position of elution of coat proteins in crude cell extracts was determined by SDS-polyacrylamide gel electrophoresis and Western blotting of column fractions. The coat protein produced by pCT1 coelutes with authentic, purified virus (Fig. 2, A and
Translation Repression by Bacteriophage MS2 Coat Protein

which (if either) of the 2 cysteine residues participates in this reaction. Replacement of either cysteine residue with serine results in reduced repressor activity. Since substitution of Cys-46 by serine results in complete loss of repression, one might be inclined to propose (as others have, Ref. 16) that Cys-46 is the residue involved in formation of the postulated covalent adduct. The assignment of either of the 2 cysteines as RNA contact sites, however, is compromised by the fact that both C46S and C101S result in apparent stability or assembly defects. One cannot tell to what extent this accounts for lost repressor activity, as opposed to direct disruption of a protein-RNA contact. Instability or improper folding could result in a gross deformity in coat protein structure. Since the repressor is apparently a dimer (20), any disruption of the ability to dimerize would also indirectly affect binding activity. Since neither of the cysteines is involved in disulfide bonding (16), it was not obvious that their substitution with serine would be so disruptive.

The potential function of the cysteine residues as sites of contact with RNA is further brought into question by the results of codon-directed mutagenesis experiments (21) that show that although some substitutions are as disruptive as serine (specifically, arginine and tryptophan), some others (phenylalanine, leucine, methionine, tyrosine, alanine, and valine) can be substituted for Cys-46 with little or no loss of repressor function. Cys-101, however, is more sensitive to substitution. Of 18 substitutions tested so far, only cysteine and arginine can be used to support normal repression. Thus, while it must be regarded as unlikely that Cys-46 is an RNA contact residue, the higher sensitivity of Cys-101 to substitution suggests that it could represent a site of interaction. The replacement of Cys-101 with arginine could establish new interactions in place of those that are lost by the removal of cysteine. More experiments are required to establish whether Cys-101 is a contact site and whether a transient covalent bond contributes to RNA binding affinity in this case.

These results also indicate that mutations of the translational operator can yield a constitutive phenotype. Plasmid pRZ6 contains an operator sequence modified in two positions to resemble the operator of the group I RNA phage GA (Ref. 17, and Fig. 1). The rules of MS2 translational operator structure predict that these changes should result in a several hundred-fold decrease in $K_c$ in vitro (3). However, only a partial loss of repression is observed when the pCT1/pRZ6 strain is compared to pUCter3/pRZ6. Perhaps coat protein exhibits a higher affinity for the mutant operator in vivo than that predicted by the in vitro binding experiments, or maybe the repressor is present in sufficient excess compared to its binding site on the hybrid replicase-lacZ mRNA to result in the unexpectedly high level of repression. Since the plasmid-produced coat protein forms capsids in vivo, it is also possible that the operator containing RNAs are packaged into virus-like particles. The formation of such complexes is probably nearly irreversible and could result in a higher apparent affinity in vivo than is observed in vitro, where conditions favoring capsid formation are avoided.

Note also that even in the absence of repressor (pUCter3), the operator mutations in pRZ6 give rise to higher levels of β-galactosidase activity than the wild-type operator sequence in pRZ5. The mutations may improve the efficiency of the replicase ribosome binding site, perhaps by changes in the Shine-Dalgarno sequence, or because of changes in RNA tertiary interactions that make the site more accessible. Caution must be exercised when interpreting the results of such experiments, since mutations which increase the inherent translational efficiency of the replicase-β-galactosidase

---

**DISCUSSION**

These results show that the two-plasmid expression system mimics the translational repression observed in MS2-infected cells. Repression of translation of the hybrid replicase-β-galactosidase sequence depends on the presence of coat protein. Repression can be reduced or eliminated either by mutation of the coat protein gene or by mutation of the translational operator. This provides a convenient means for the isolation of a variety of coat mutants altered in their ability to bind wild-type or mutant operators.

Uhlenbeck and his colleagues (4) have presented evidence suggesting that a coat protein sulfhydryl group is involved in the formation of a transient covalent bond with the pyrimidine ring at position -5 in the translational operator. The results of these experiments do not permit us to conclude

---

**FIG. 5.** Electron micrographs of MS2 virus and partially purified virus-like particles produced in CSH4i(pCT1).
mRNA might mimic the operator-constitutive phenotype without actually affecting the strength of the coat protein-operator interaction. In this case, however, comparison of the ratios of derepressed to repressed enzyme levels reveals a clear loss of repression in pRZ6 compared to pRZ5 (50-fold versus 7-fold). The level of derepression is easily distinguished from wild-type by colony color on X-gal plates, making it possible to isolate mutations in the coat protein gene that suppress defects in the operator sequence. The ability to quickly isolate milligram quantities of plasmid-produced coat protein will facilitate the biochemical analysis of coat protein variants produced by genetic means.

Acknowledgment—I wish to express gratitude to David Bear for electron microscopy and discussions.

REFERENCES
1. Bernardi, A., and Spahr, P. F. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 3033-3037
2. Romaniuk, P. J., Ollvary, P., Wu, H.-N., Stormo, G., and Uhlenbeck, O. C. (1987) Biochemistry 26, 1563-1568
3. Uhlenbeck, O. C., Carey, J., Romaniuk, P. J., Lowary, P. T., and Beckett, D. (1983) J. Biomol. Struct. & Dyn. 1, 539-552
4. Romaniuk, P. J., and Uhlenbeck, O. C. (1985) Biochemistry 24, 4239-4244
5. Devos, R., van Emmelo, J., Contreras, R., and Fiers, W. (1979) J. Mol. Biol. 128, 596-619
6. Min Jou, W., Haegeman, G., Ysebaert, M., and Fiers, W. (1972) Nature 237, 82-88
7. Fiers, W., Min Jou, W., Duerinck, F., Haegeman, G., Merregaert, J., Min Jou, W., Raeymaekers, A., Volckaert, G., Ysebaert, M., Van de Kerchove, J., Nolf, F., and Van Montagu, M. (1975) Nature 256, 279-278
8. Fiers, W., Min Jou, W., Duerinck, F., Haegeman, G., Iserentant, D., Merregaert, J., Min Jou, W., Molenaars, F., Raeymaekers, A., Van den Berghe, A., Volckaert, G., and Ysebaert, M. (1976) Nature 260, 500-507
9. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103-119
10. Kunkel, T. A., Roberts, J. D., and Zalkin, R. A. (1987) Methods Enzymol. 154, 367-382
11. Scheuermann, R. H., and Echols, H. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7747-7751
12. Chung, A. C. Y., and Cohen, S. N. (1978) J. Bacteriol. 134, 1141-1156
13. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Laemmli, U. K. (1970) Nature 227, 680-685
15. Burnette, W. N. (1981) Anal. Biochem. 112, 195-203
16. Berzin, V. M., Tsimanis, A. Y., Gren, E. Y., Zaikowski, W., and Shafirski, P. (1981) Bioorg. Khim. 7, 894-899
17. Inokuchi, Y., Takashi, R., Hirose, T., Inayama, S., and Jacobson, A. B. (1986) J. Biochem. (Tokyo) 99, 1169-1180
18. Valegard, K., Torsten, U., Montelius, I., Strandberg, B., and Fiers, W. (1986) J. Mol. Biol. 190, 591-595
19. Fukuda, A. A., Young, V. B., and Sauer, R. T. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8829-8833
20. Beckett, D., and Uhlenbeck, O. C. (1989) J. Mol. Biol. 204, 927-938
21. Peabody, D. S. (1989) Nucleic Acids Res. 17, 6017-6027
Translational repression by bacteriophage MS2 coat protein expressed from a plasmid. A system for genetic analysis of a protein-RNA interaction.

D S Peabody

J. Biol. Chem. 1990, 265:5684-5689.

Access the most updated version of this article at http://www.jbc.org/content/265/10/5684

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

Click here 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/265/10/5684.full.html#ref-list-1