Role of Malignant Hyperthermia Domain in the Regulation of Ca\(^{2+}\) Release Channel (Ryanodine Receptor) of Skeletal Muscle Sarcoplasmic Reticulum*

(Received for publication, May 29, 1996, and in revised form, July 10, 1996)

Francesco Zorzato‡, Paola Menegazzi, Susan Treves, and Michel Ronjat‡§

From the Institute of General Pathology, University of Ferrara, Via Borsari 46, 44100 Ferrara, Italy, the §Laboratoire de Biophysique Moleculaire et Cellulaire, URA 520 du CNRS, CEA/CENG, Department de Biologie Moleculaire et Structurale, 17 rue des Martyrs, 38054 Grenoble Cédex 9, France

A fusion protein encompassing Gly\(^{341}\) of the skeletal muscle ryanodine receptor was used to raise monoclonal antibodies; epitope mapping demonstrates that monoclonal antibody 419 (mAb419) reacts with a sequence a few residues upstream from Gly\(^{341}\). The mAb419 was then used to probe ryanodine receptor (RYR) functions. Our results show that upon incubation of triads vesicles with mAb419 the Ca\(^{2+}\) release rate at pCa 8 was increased. Equilibrium evaluation of \([^{3}H]\)ryanodine binding at different [Ca\(^{2+}\)] indicates that mAb419 shifted the half-maximal [Ca\(^{2+}\)] for stimulation of ryanodine binding to lower value (0.1 versus 1.2 \(\mu\)M). Such functional effects may be due to a direct action of the Ab on the Ca\(^{2+}\) binding domain of the RYR or to the perturbation by the Ab of the intramolecular interaction between the immunopositive region and regulatory domain of the RYR. The latter hypothesis was tested using the optical biosensor BIAcore (Pharmacia Biotech Inc.): we show that the immunopositive RYR polypeptide is able to interact with the native RYR complex. Ligand overlays with immunopositive digoxigenin-RYR fusion protein indicate that such an interaction might occur with a calmodulin binding domain (defined by residues 3010–3225) and with a polypeptide defined by residues 799–1172. In conclusion our results suggest that the stimulation by the mAb419 of the RYR channel activity is due to the perturbation of an intramolecular interaction between the immunopositive polypeptide and a Ca\(^{2+}\) regulatory site probably corresponding to a calmodulin binding domain.

The skeletal muscle sarcoplasmic reticulum is an intracellular membrane compartment that controls the myoplasmic Ca\(^{2+}\) concentration, thereby playing an important role in the excitation-contraction coupling mechanism (Endo, 1985; Franzini-Armstrong, 1980). Skeletal muscle contraction is triggered by release of Ca\(^{2+}\) from sarcoplasmic reticulum terminal cisternae (Somlyo et al., 1985) via a Ca\(^{2+}\) release channel which is believed to be the ryanodine receptor (RYR).\(^{1}\) Rios and Pizzaro, 1991), a large homotetrameric oligomer made up of four 565-kDa subunits (Pessah et al., 1986; Inui et al., 1987; Smith et al., 1988; Lai et al., 1988; Takeshima et al., 1989; Zorzato et al., 1990; Nakai et al., 1990). Recently it has been found that calmodulin both activates and inhibits the RYR Ca\(^{2+}\) channel, depending upon the free [Ca\(^{2+}\)]; at submicromolar Ca\(^{2+}\) calmodulin activates the channel, while at micromolar Ca\(^{2+}\) calmodulin inhibits channel activity (Tripathy et al., 1995; Buratti et al., 1995). The activation of the RYR Ca\(^{2+}\) channel by calmodulin is accompanied by the binding of calmodulin to the domain encompassed between residues 3010–3225 (Buratti et al., 1995). An Arg to Cys substitution at position 615 in the primary structure of the RYR Ca\(^{2+}\) channel from malignant hyperthermia (MH)-susceptible pigs has been correlated with abnormalities in the sensitivity of Ca\(^{2+}\) channel activity, indicating that mutations in the RYR are potential molecular defects underlying MH (Michelson et al., 1988; Fill et al., 1990, 1991; Fuji et al., 1991). Since the description of the Arg\(^{615}\) → Cys mutation in the MH pigs, eight other mutations in the RYR have been shown to cosegregate with the MH phenotype in human pedigrees. Six out of these eight mutations are clustered between Arg\(^{613}\) and Tyr\(^{522}\). However, except for the mutation Arg\(^{615}\) → Cys, to date no biochemical evidence has been obtained linking these mutations to altered functional properties of the human RYR.

In the present report we raised a monoclonal antibody (mAb419) against a skeletal muscle RYR domain encompassing Gly\(^{341}\), a residue that has been shown to mutate in 10% of human MH pedigrees (Quane et al., 1994). We found that this mAb increases both Ca\(^{2+}\) release rate and shifted the Ca\(^{2+}\) dependence ryanodine binding to lower values. Biospecific interaction analysis revealed that the immunopositive polypeptide interacts with the native RYR complex. Ligand overlay experiments indicate that this interaction might occur via the calmodulin binding domains of the RYR defined by residues 3010–3225.

EXPERIMENTAL PROCEDURES

Materials

Nitrocellulose was from Schleicher and Schuell; restriction enzymes, DNA-modifying enzymes, calmodulin, digoxigenin-3-O-methylcarbonyl-\(\epsilon\)-aminocaproic acid N-hydroxysuccinimide ester, anti-digoxigenin peroxidase-conjugated Ab, and BM chemiluminescence were from Boehringer Mannheim; anti-mouse IgG, protein molecular weight markers, and dansyl-calmodulin were from Sigma; the blucerscript cloning vector was from Stratagene; \(^{45}\)Ca\(^{2+}\) and \([^{3}H]\)ryanodine were from DuPont NEN; all ther chemicals were reagent grade.

Methods

Preparation of Sarcoplasmic Reticulum Fractions—Terminal cisterna fraction were obtained from the white skeletal muscle of New Zealand rabbits as described by Saito et al. (1984). Triads were purified according to Marty et al. (1994).

DNA Manipulation and Production of Fusion Proteins—DNA manip-
ulations were carried out according to standard protocols as described in Maniatis et al. (1989). To cover the entire RYR coding sequence, we constructed several fusion proteins (Koerner et al., 1991). The details of the construction of RYR fusion proteins have been described previously (Treves et al., 1993; Menegazzi et al., 1994). The EcoRI III/mung nuclease digestion of the 3' end of the cDNA encoding the RYR fusion protein PC25 was carried out as described previously (Zorzato et al., 1990).

**SDS-Polyacrylamide Gel Electrophoresis and Immunological Techniques**—Slab gel electrophoresis was carried out as described by Laemmli (1970). Western blots of bacterial extracts were carried out overnight as described by Gershoni et al. (1985). Indirect immunoenzymatic staining of Western blots was carried out as described by Young et al. (1985) and detailed by Treves et al. (1993). mAb419 was extracted from culture medium first by (NH₄)₂SO₄ precipitation and further purified by using DEAE-cellulose anion-exchange column chromatography (Harlow and Lane, 1988). The immunoglobulin fraction was then dialyzed overnight against phosphate-buffered saline at 4°C.

**45Ca²⁺ Release and Ryanodine Binding Assay**—Triad vesicles (1 mg/ml) were incubated in the presence or in the absence of mAb419 (Ab/HSR ratio of 1) for 2 h at 37°C. 45Ca²⁺ loading was carried out for 30 min at room temperature in the presence of 2.5 mM CaCl₂, 75 mM KCl, 25 mM MES, pH 7.0. Rapid kinetic 45Ca²⁺ release was measured by using a rapid filtration apparatus (Biologic, Claix, France) as described previously (Marty et al. 1988). [³H]Ryanodine binding was measured as described previously (Marty et al., 1994) at an Ab/heavy sarcoplasmic reticulum ratio of 1 in a solution containing 25 mM MES, pH 7.0, 0.15 mM KCl, 2 mM EGTA, and 50 nM [³H]ryanodine, and various concentration of Ca²⁺.

**Real Time Surface Plasmon Resonance Recording**—For real time binding experiments a BIAcore biosensor system (Pharmacia Biotech, Inc.) was used. All experiments were performed at 25°C. Between injections the sensor chip was continuously washed with 10 mM HEPES, pH 7.5, 150 mM NaCl, 3.4 mM EDTA, 0.005% BIAcore surfactant P20 (Pharmacia). The fusion protein 19E was directly coupled through its amino groups to the sensor surface activated by N-hydroxysuccinimide and N-ethyl-N-(dimethylaminopropyl)carbodiimide according to the manufacturer's instruction. The remaining N-hydroxysuccinimide groups were then inactivated with 1 mM ethanolamine.

**Ligand Overlays**—Fusion protein 19E was electroeluted from SDS-polyacrylamide gel and then labeled with digoxigenin-3-O-methylcarnbonyl-e-aminoacrylic acid N-hydroxysuccinimide ester according to manufacturer's instructions. Overlays with digoxigenin-fusion protein 19E and -calmodulin were carried out as described by Menegazzi et al. (1994). 4Ca²⁺ overlay was carried out as described by Maruyama et al. (1984).

**RESULTS AND DISCUSSION**

**Characterization of mAb419**—We raised monoclonal Abs (mAb419) against a fusion protein (number 19) encompassing residues 281–620 of the rabbit skeletal muscle RYR protomer. The monoclonal Ab 419 reacted strongly with the protein band corresponding to the RYR protomer and with its own Ag (Fig. 1A, lanes 1b and 2b, respectively). To map the RYR immunopositive peptide within fusion protein 19, we made a set of constructs and probed the respective fusion proteins with mAb419. Fig. 1B shows that the immunological reactivity of the monoclonal antibody was retained only by fusion proteins 19A and 19E. On the contrary the Ab did not recognize fusion proteins 19C and 19D, suggesting that the sequence surrounding the break point between 19C and 19D is crucial to confer the immunoreactivity to mAb419. Of interest, the break point, which abolishes immunological reactivity occurs in correspondence of Met^{325} i.e. few residues upstream Gly^{341}.

**Effect of mAb419 on the Functional Properties of the RYR Ca^{2+} Channel**—Triad vesicles were incubated with mAb419 and then passively loaded with 4Ca²⁺. Rapid 4Ca²⁺ release kinetics were monitored at pCa 8. As shown by Fig. 2A, in the presence of mAb419 the rate constant of Ca²⁺ release (k = 32.1 s⁻¹) was increased with respect to control experiments (k = 12 s⁻¹). Activation of the Ca²⁺ release rate (k = 4.2 s⁻¹ versus k = 72.4 s⁻¹) was also observed when the experiments were carried out at pCa 4.5 (Fig. 2B). To further confirm the effect of the mAb419 on the functional properties of RYR, we measured the equilibrium binding of [³H]ryanodine at different pCa. Fig. 2C shows that the Ca²⁺ dependence of [³H]ryanodine binding displays a bell-shaped curve both in the presence and in the absence of mAb419. However, the half-maximal [Ca²⁺] for stimulation of [³H]ryanodine binding was significantly lower in the presence of mAb419 (0.1 versus 1.5 μM). No significant change for inhibition of ryanodine binding by high (Ca²⁺) was observed, indicating the specificity of the effect of mAb419. The increase of Ca²⁺ sensitivity of both 4Ca²⁺ release and ryanodine binding rates in the presence of mAb419 could be due to a direct interaction with a domain involved in Ca²⁺ binding or to a modification of a Ca²⁺-dependent regulatory site, which is adjacent in the three-dimensional structure but may be distant in the linear sequence. To discriminate between these two
possibilities, we examined whether the set of RYR fusion proteins we produced encompass Ca$^{2+}$ binding sites. In agreement with previous results, $^{45}$Ca$^{2+}$ overlay of fusion proteins 19, 19A-19E indicates that none of them contain Ca$^{2+}$ dependent allosteric sites (not shown). Thus, the effect of the mAb on Ca$^{2+}$ dependence of ryanodine binding could be due to the perturba-

**FIG. 2.** Effect of mAb419 on the functional activity of the RYR. Triad vesicles (1 mg/ml) were incubated in the absence (filled squares) or presence (filled circles) of mAb419 (Ab: membrane proteins = 1, w/w). Mean ± S.D. (n = 3) are shown. Ca$^{2+}$-induced Ca$^{2+}$ release experiments: triad vesicles were then loaded with $^{45}$Ca and Ca$^{2+}$-induced Ca$^{2+}$ release measured at pCa 8 (A) and pCa 4.5 (B) as described under “Materials and Methods.” $^{45}$Ca$^{2+}$ loading: 48 + 7 and 65 + 8 nmol/mg p (A), 62 ± 7 and 58 ± 13 nmol/mg protein (B), in the absence and in the presence of mAb419, respectively. C, [H]$^3$Hryanodine binding on triad vesicles; Ca$^{2+}$ dependence of equilibrium [H]$^3$Hryanodine binding was measured as described under “Materials and Methods.”

**FIG. 3.** Characterization of the binding of fusion protein 19E with the purified RYR using surface plasmon resonance detection. Fusion protein 19E was immobilized on the sensor chip surface as described under “Materials and Methods.” The traces are representative of four different experiments. A represents the interaction of mAb419 (5.12 μg) with the immobilized fusion protein 19E. B represents the interaction of the purified RYR with immobilized fusion protein 19E. Curves are as follows: curve A, 1.6 μg of RYR; curve B, 1.6 μg of RYR plus 1.5 μg of fusion protein 19E; curve C, 7.5 μg of RYR plus 15 μg of fusion protein 19E. After each binding experiment, the sensor chip was regenerated as described under “Materials and Methods.” The specific binding signal shown was obtained by subtracting the signal measured in the absence of immobilized fusion proteins from the signal in the presence of the fusion protein. C, effect of preincubation of RYR with fusion protein 19E. The RYR complex was first preincubated for 15 min at room temperature with RYR complex and then its interaction with the fusion protein coupled to sensor chip was monitored as described in the legend for B. Curves are as follows: curve A, 1.6 μg of the RYR; curve B, 1.6 μg of RYR plus 1.5 μg of fusion protein 19E; curve C, 7.5 μg of RYR plus 15 μg of fusion protein 19E.
tion by the Ab of a regulatory sequence adjacent to the Ab’s epitope in the three-dimensional structure. Such a regulatory domain could be localized in the RYR itself or on a protein associated with the RYR complex.

**Molecular Interaction of Immunopositive RYR Fusion Protein 19E**—To investigate potential molecular interactions of the immunopositive fusion protein 19E with protein components of the Ca\(^{2+}\) release machinery, we immobilized fusion protein 19E onto the dextran matrix coating the gold sensor chip, and its interaction with the purified RYR was monitored using the optical biosensor BIAcore (Pharmacia). Fig. 3A shows the sensorgram representing real time binding of mAb419 to the immobilized 19E RYR fusion protein; the signal increased and reached a plateau in few hundred seconds, indicating that the immobilization step did not modify the immunological properties of the fusion protein. In the next set of experiments, we analyzed the interaction of the purified RYR complex with the RYR fusion protein 19E. To prevent nonspecific binding of the purified RYR complex to the sensor chip we first injected 10 \(\mu\)l of 1 mg/ml bovine serum albumin and then the purified RYR complex into the flow cell. Injection of bovine serum albumin produced no signal (not shown). The sensorgrams in Fig. 3B show the real time interaction of the immobilized 19E RYR fusion protein with increasing concentrations of purified RYRs. As can be seen, the resonance unit response of the 19E-coupled sensor chip is dose-dependent and approaches saturation after injection of 4.15 \(\mu\)g of purified RYR complex. The interaction between the RYR and the immobilized fusion protein could be inhibited by preincubation of the RYR complex with free fusion protein 19E (Fig. 3C). These results clearly indicate that a specific binding between the purified RYR complex and fusion protein 19E takes place.

To identify the domain(s) of the RYR involved in the interaction with fusion protein 19E, we performed overlays on Western blots of fusion proteins covering the entire length of the RYR were separated on 7.5 or 10% SDS-PAGE, blotted onto nitrocellulose, and colored with Ponceau red (lanes a) or subjected to ligand overlay (lanes b). Fifty \(\mu\)l of bacterial extracts were loaded per lane. Arrows indicate the RYR fusion proteins. *Upper panel*, fusion protein production strategy. The numbering is positive beginning the first nucleotide of initiator methionine. The underlying segments indicate the cDNA fragments cloned into the bacterial expression vectors.

![Identification of the RYR domains involved in the interaction with fusion protein 19E](image)

**Fig. 4. Identification of the RYR domains involved in the interaction with fusion protein 19E.** Ligand overlay was carried out at 100 nm digoxigenin-fusion protein 19E. Terminal cisternae or fusion proteins covering the entire length of the RYR were separated on 7.5 or 10% SDS-PAGE, blotted onto nitrocellulose, and colored with Ponceau red (lanes a) or subjected to ligand overlay (lanes b). Fifty \(\mu\)l of bacterial extracts were loaded per lane. Arrows indicate the RYR fusion proteins. *Upper panel*, fusion protein production strategy. The numbering is positive beginning the first nucleotide of initiator methionine. The underlying segments indicate the cDNA fragments cloned into the bacterial expression vectors.

![Identification of the domain of fusion proteins 28 and 1 involved in the interaction with fusion protein 19E](image)

**Fig. 5. Identification of the domain of fusion proteins 28 and 1 involved in the interaction with fusion protein 19E.** A, conditions and experiments are the same as in Fig. 5, except that the overlay was carried out on the fusion proteins 28 and with a fusion protein 28 carrying a deletion at its COOH terminus. *Lanes*: a, Ponceau red staining of bacterial extracts; b, fusion protein overlay; c, calmodulin overlay. *Arrows* indicate fusion protein; *B*, conditions and experiments are the same as in A. Overlays were carried out on different domains of fusion protein 1. *Lanes*: a, Ponceau red staining of bacterial extracts; b, fusion protein overlay. *Arrows* indicate fusion proteins.
In conclusion the data obtained in this study demonstrate that an anti-RYR mAb directed against an epitope localized few residues upstream Gly\(^{341}\) activates both Ca\(^{2+}\) release and \(^{3}H\)ryanodine binding activity. Interestingly, this apparent increase of the Ca\(^{2+}\) sensitivity of the RYR in the presence of mAb419 is similar to what has been observed previously with triad vesicles from MH-susceptible pigs (Michelson et al., 1988). Nevertheless Ca\(^{2+}\) overlay experiments indicate that the fusion proteins 19 and 19A-19E do not display Ca\(^{2+}\) binding. Therefore the effect of mAb419 suggests that the sequence corresponding to fusion protein 19E interact with another region of the RYR directly involved in the regulation of the channel by Ca\(^{2+}\). Real time surface plasmon resonance (BIA-core technique) indicates that the fusion protein 19E is able to interact with the purified native RYR complex. As to the sequence(s) involved in the intramolecular interaction between the fusion protein 19E and the RYR complex two polypeptides were identified: the first is localized at the NH\(_2\)-terminal portion of the RYR protomer and is defined by residues 799-1172, the second is a calmodulin binding domain that is able to interact with calmodulin at low [Ca\(^{2+}\)] (Menegazzi et al., 1994). We also show that a modification of the calmodulin binding site, which leads to the loss of calmodulin binding, also brings a functional role for the region of the RYR encompassing Gly\(^{341}\); it is tempting to think that this residue plays a crucial role in the intramolecular link between sequences encompassed by fusion protein 19E and calmodulin binding domains of the RYR. The abnormalities of the RYR in MH susceptible individuals could be due to a modification of this intramolecular interaction, which may in turn lead to an increase in Ca\(^{2+}\) sensitivity. However, one should also consider that 19E interacts with another region of the RYR defined by residues 799-1172 as well as with a 110-kDa peptide present in terminal cisternae vesicles. These interactions could also play an important role in the regulation of Ca\(^{2+}\) release from the RYR.

**REFERENCES**

Buratti, R., Prestipino, G., Menegazzi, P., Treves, S., and Zorzato, F. (1995) *Biophys. Biochem. Res. Commun.* 213, 1941–1945.

Endo, M. (1985) *Curr. Top. Membr. Transp.* 25, 181–229.

Fill, M., Coronado, R., Michelson, J. R., Vilven, J., Ma, J., Jacobson, B. A., and Louis, C. F. (1990) *Biophys. J.* 59, 471–475.

Fill, M., Stefanini, E., and Nelson, T. E. (1991) *Biophys. J.* 59, 1085–1090.

Frantzi-Armstrong, C. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 39, 2403–2409.

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O’Brien, P. J., and MacLennan, D. H. (1991) *Science* 253, 448–451.

Gershoni, J. M., Davis, F. E., and Palade, G. E. (1985) *Anal. Biochem.* 144, 32–40.

Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual*, pp. 286–299, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Inui, M., Saito, A., and Fleischer, S. (1987) *J. Biol. Chem.* 262, 1740–1747.

Koerner, T. J., Hill, J. E., Myers, A. M., and Tzagoloff, A. (1991) *Methods Enzymol.* 194, 477–490.

Laemmli, U. K. (1970) *Nature* 227, 680–685.

Lai, F. A., Erickson, H. P., Rosseau, E., Liu, Q. Y., and Meissner, G. (1988)*Nature* 331, 315–319.

Mannias, T., Fritsh, E. F., and Sambrook, J. (1989) *Molecular Cloning: A Laboratory Manual*, Second Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Marty, I., Robert, M., Villaz, M., De Jongh, R. S., Lai, Y., Catterall, W. A., and Ronjat, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 2270–2274.

Maruyama, K., Mikawa, T., and Ebashi, S. (1984) *J. Biochem. (Tokyo)* 95, 511–519.

Menegazzi, P., Larini, F., Treves, S., Guerrini, R., Quadroni, M., and Zorzato, F. (1994) *Biochemistry* 33, 9078–9084.

Michelson, J. R., Gallant, E. M., Litterer, L. A., Johnson, K. M., Rempel, W. C., and Louis, C. F. (1988) *J. Biol. Chem.* 263, 9310–9315.

Moutin, M. J., and Dupont, Y. (1988) *J. Biol. Chem.* 263, 4228–4235.

Nakai, J., Imagawa, T., Hakomata, Y., Shigekawa, M., Takeshima, H., and Numa, S. (1990) *FERS Lett.* 271, 169–177.

Pessah, I. N., Francini, A. O., Scales, D. J., Weterhouse, A. L., and Casida, J. E. (1985) *J. Biol. Chem.* 260, 8483–8488.

Quane, K. A., Keating, K. E., Manning, B. M., Healy, J. M., Monsier, K., Lunnardi, J., and McCarthy, T. V. (1994) *Hum. Mol. Gen.* 3, 471–476.

Rios, E., and Pizarro, G. (1991) *Physiol. Rev.* 71, 849–908.

Saito, A., Seiler, S., Chu, A., and Fleisher, S. (1984) *J. Cell Biol.* 99, 975–985.

Smith, J. S., Imagawa, T., Ma, J., Fill, M., Campbell, K. P., and Coronado, R. (1988) *J. Gen. Physiol.* 92, 1–26.

Somlyo, A. V., McLellan, G., Gonzales-Serratos, H., and Somlyo, A. P. (1985) *J. Biol. Chem.* 260, 6801–6807.

Takeshima, H., Nishimura, S., Matsumoto, H., Ishida, K., Kangawa, N., Minamino, H., Matsuo, M., Ueda, M., Hanakos, M., Hirose, T., and Numa, S. (1989) *Nature* 339, 449–455.

Tripathy, A., Xu, L., Mann, G., and Meissner, G. (1995) *Biophys. J.* 69, 103–119.

Treves, S., Chiozzi, P., and Zorzato, F. (1993) *Biochem. J.* 291, 757–763.

Young, R. A., Bloom, B. R., Grosskinsky, G. M., Ivanyi, J., Thomas, D., and Davis, R. W. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82, 2583–2587.

Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N. M., Lai, F. A., Meissner, G., and MacLennan, D. H. (1990) *J. Biol. Chem.* 265, 2244–2256.
Role of Malignant Hyperthermia Domain in the Regulation of Ca^{2+} Release Channel (Ryanodine Receptor) of Skeletal Muscle Sarcoplasmic Reticulum
Francesco Zorzato, Paola Menegazzi, Susan Treves and Michel Ronjat

J. Biol. Chem. 1996, 271:22759-22763.
doi: 10.1074/jbc.271.37.22759

Access the most updated version of this article at http://www.jbc.org/content/271/37/22759

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 28 references, 11 of which can be accessed free at http://www.jbc.org/content/271/37/22759.full.html#ref-list-1