Molecular Cloning and Expression of the Regulatory (RG1) Subunit of the Glycogen-associated Protein Phosphatase*

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DNA clones encoding the glycogen-binding (RG1) subunit of glycogen-associated protein phosphatase were isolated from rabbit skeletal muscle agt11 cDNA libraries. Overlapping clones provided an open reading frame of 3327 nucleotides that predicts a polypeptide of 1109 amino acids with a molecular weight of 124,257. Northern hybridization of rabbit RNA identified a major mRNA transcript of 7.5 kilobases present in skeletal, diaphragm, and cardiac muscle, but not in brain, kidney, liver, and lung. Southern analysis of rabbit genomic DNA digested with various restriction endonucleases gave rise to a single hybridizing fragment, suggesting that a single gene is present. Expression of the complete RG1 subunit coding sequence in Escherichia coli generated a protein of apparent molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis of approximately 160,000, similar to the size of the polypeptide detected by Western immunoblot in rabbit skeletal muscle extracts. The RG1 subunit shares significant homology with the Saccharomyces cerevisiae GAC1 gene product which is involved in activation of glycogen synthase and glycogen accumulation. The homology with GAC1 substantiates the role of this enzyme in control of glycogen metabolism. Hydropathy analysis of the RG1 subunit amino acid sequence revealed the presence of a hydrophobic region in the COOH terminus, suggesting a potential association with membranes. This result suggests that the same phosphatase regulatory component may be involved in targeting the enzyme both to membranes and to glycogen.

Protein phosphorylation is a major mechanism by which many cellular functions are regulated. The co-ordinated con-

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1 The abbreviations used are: Rcl, glycogen-binding regulatory subunit of type 1 protein phosphatase. This protein had previously been designated "G subunit," causing some confusion with GTP binding proteins. We propose the present nomenclature as a means to avoid this confusion. In addition, other type 1 phosphatase regulatory subunits can be named in a parallel fashion, RSR and RM1 for the inhibitor-2, (b) glycogen-binding or RG1 subunit, (c) sarcoplasmic reticulum-binding subunit, and (d) a putative myosin-binding subunit. A recent report has shown that the properties of the sarcoplasmic reticulum-associated phosphatase in rabbit skeletal muscle are similar to those of the glycogen-bound phosphatase, suggesting that the RG1 subunit might play a dual role in targeting type 1 phosphatase to two different subcellular locations, glycogen and membranes.

Up to 60% of rabbit skeletal muscle phosphorylase phosphatase is associated with glycogen (27, 28). This glycogen-bound protein phosphatase was first identified and purified as a 137-kDa heterodimer, consisting of a 37-kDa catalytic subunit and a 103-kDa regulatory component, the RG1 subunit (20). However, the RG1 subunit is extremely sensitive to proteolysis. More recent analysis by Western immunoblotting

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indicated that the intact Rcl subunit has a M_, of 160,000–170,000 (28, 29). Its function appears to be targeting the phospha-
tase to the glycogen particle, where several of the en-
as, such as glycogen synthase and phosphorylase ki-
ze, involved in glycogen metabolism are located. The Rcl
subunit is phosphorylated in vitro by the cAMP-dependent protein kinase at two sites (29). Phosphorylation of site 2 has
been proposed to cause dissociation of the catalytic subunit
(30). Studies from our (31) and Cohen's (32) laboratories also
revealed a complex multisite phosphorylation of the Rcl sub-
unit. Phosphorylation by cAMP-dependent protein kinase
formed the recognition sites for other protein kinases such as
glycogen synthase kinase-3 and casein kinase II. The cAMP-
dependent protein kinase sites and one of the glycogen syn-
thease kinase-3 sites (33) have been shown to be phosphoryl-
ad in vivo. Epinephrine is reported to enhance significantly the
phosphate content of site 2 (33), whereas insulin leads to
increased phosphorylation of site 1 (34).

To investigate the native structure and the role of the Rcl
subunit in the regulation of the glycogen-associated phospha-
tase we have undertaken the molecular cloning of cDNAs
coding for this subunit. This paper reports the first isolation
and characterization of cDNA clones encoding the Rcl sub-
unit. The choice of tissue and the temperature dependence of the entire trans-
lated region, the tissue specific distribution, and the expres-
sion of the coding sequences in Escherichia coli are presented.

**EXPERIMENTAL PROCEDURES**

**Materials**—The bacteriophage T7 polymerase expression system
was generously provided by Dr. F. W. Studier (Brookhaven National Laboratories). Oligonucleotides were synthesized in an Applied Bio-
systems DNA synthesizer model 380A. Restriction and other DNA
modifying enzymes, M13 vectors, and agarose were purchased from
Bethesda Research Laboratories. GeneScribe-Z vector, pTZ19U, for
digestion, modified enzymes, M13 vectors, and agarose were purchased from
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**Sequence Determination of cDNA Clones**—Two rabbit
skeletal muscle random hexamer-primed Agt11 cDNA libraries con-
taining, respectively, 10^6 and 10^7 cDNA inserts were subcloned into the GeneScribe-Z vector, pTZ19U, for

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**Construction of GpET-8C Expression Vector**—Three cDNA clones,
HX 1-1 (895 bp), HX 1-2 (1412 bp), and HX 1-2 (2561 bp), were used to assemble the entire coding sequence. The 341-bp EcoRI-BglII
fragment containing the 5'-most sequences. The resulting DNA fragment was
assembled into plasmid pET-8C cleaved at NcoI

**Isolation and Sequencing of Genomic DNA**—Rabbit genomic DNA (20 μg)
digested with the restriction enzymes EcoRI, HindIII, and XhoI
and was separated on a 1% agarose gel and transferred to a nitrocellulose
membrane. Following prehybridization at 65 °C in a solution contain-
ing 10 × Denhardt's, 5 × SSPE, 0.05% sodium pyrophosphate, 0.1% SDS, and 0.1 mg/ml Torula RNA, the membrane was hybridized with the
599-bp cDNA insert of clone HX 1-1 labeled with the nick trans-
laction method (1 × 10^6 cpm/ml) (46). The membrane was washed at two temperatures: 68 °C in 6 × SSC, 0.1% SDS, and
0.05% sodium pyrophosphate followed by a further wash at 68 °C in 2 × SSC for 30 min.

**Primer Extension**—A 19-mer synthetic oligonucleotide comple-
mentary to residues 61–79 of rabbit Rcl cDNA was used as a primer.

**Southern Analysis of Genomic DNA**—Rabbit genomic DNA (20 μg)
digested with the restriction enzymes EcoRI, HindIII, and XhoI
and was separated on a 1% agarose gel and transferred to a nitrocellulose
membrane. Following prehybridization at 65 °C in a solution contain-
ing 10 × Denhardt's, 5 × SSPE, 0.05% sodium pyrophosphate, 0.1% SDS, and 0.1 mg/ml Torula RNA, the membrane was hybridized with the
599-bp cDNA insert of clone HX 1-1 labeled with the nick trans-
laction method (1 × 10^6 cpm/ml) (46). The membrane was washed at two temperatures: 68 °C in 6 × SSC, 0.1% SDS, and
0.05% sodium pyrophosphate followed by a further wash at 68 °C in 2 × SSC for 30 min.

Fig. 1. R<sub>c11</sub> subunit DNA clones. A partial restriction map and all the clones used for DNA sequence analysis are shown. HX denotes clones isolated from a random hexamer-primed rabbit skeletal muscle λgt11 cDNA library and DT clones obtained from an oligo(dT)-primed library. GG10-1 was a clone isolated from a genomic library. The black bar indicates the coding region; thick lines indicate regions that were sequenced in both directions; thin lines indicate regions sequenced in one direction; dashed lines indicate regions not sequenced; and the dotted line indicates a portion of an intron in the genomic DNA clone.

by adding 0.5 or 1.0 mM isopropyl-β-D-thiogalactopyranosidase (IPTG), and the cell growth was continued for 3 h at 30 °C. Cells were harvested by centrifugation at 7,000 × g for 15 min and resuspended in 30 volumes of buffer containing 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM TLCK, 2 mM benzamidine, 10 μg/ml leupeptin, 50 mM β-mercaptoethanol, and 1% Triton X-100. After freezing at −80 °C overnight, the cells were thawed and sonicated for 20 s twice. The lysate was centrifuged at 9,000 × g for 20 min. A sample of 1.4 μl each of the whole cell lysate and the Triton-soluble fraction were analyzed by SDS-PAGE according to Laemmli (48) and by Western immunoblotting (49).

Western Blot Analysis—A synthetic peptide KPFGSPQPS-RGGSESEEEVYV surrounding the CAMP-dependent protein kinase phosphorylation site on the R<sub>c11</sub> subunit was synthesized and used to raise antibodies (anti-R<sub>c11</sub>) in guinea pigs. The antibodies were affinity-purified on Sepharose 4B coupled to the peptide. Rabbit skeletal muscle was homogenized in 4 volumes of 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 0.5 mM benzamidine, 0.1 mM TLCK, 10 μg/ml leupeptin and centrifuged for 20 min at 10,000 × g. A 1-μl sample of the rabbit skeletal muscle soluble extract and samples of E. coli extract prepared as described above were subjected to 7.5% SDS-PAGE. For immunoblotting, gels were equilibrated for 20 min in 20% methanol, 10% acetic acid, and subjected to ice-cooled transverse electrophoresis at 100V for 2 h. The nitrocellulose filter was blocked overnight at room temperature in 5% powdered milk in PBS-T (20 mM sodium phosphate, pH 7.4, 118 mM sodium chloride, and 0.1% Tween 20) and then incubated for 2 h with anti-R<sub>c11</sub> antibody (5 μg/ml in PBS-T). Bound antibodies were detected by incubating the filter for 1 h in PBS-T containing 0.2 μCi/ml of 32P-protein A. After removal of unreacted protein A the filter was subjected to autoradiography.

RESULTS

Isolation and Characterization of cDNA and Genomic DNA Clones—The initial screening of the rabbit skeletal muscle random hexamer-primed λgt11 cDNA library identified two positive clones that hybridized with both oligonucleotide probes G-COMP1 and G-COMP2. Nucleotide sequencing confirmed that clone HX 1-1 (995 bp) coded for the available amino acid sequences (50) of the R<sub>c11</sub> subunit polypeptide. This labeled cDNA fragment was used to rescreen the original filters and additional 160,000 plaque-forming units. Clones isolated from this screen were used for subsequent screening of another random hexamer-primed and an oligo(dT)-primed cDNA libraries. A total of 630,000 independent recombinants were screened, and 74 positive clones were identified, out of which 23 were characterized. Complete nucleotide sequences were determined from six clones (HX 1-1, 1-2, 5-1, 11-1, 13-2, and DT 6-1), whereas partial sequences were obtained from other clones including HX 11-2, 15-1, 16-1, 18-1, 18-2, 18B-1, 21-1, and DT 3-1 (Fig. 1). These overlapping clones provided a combined sequence with an open reading frame of 3317 nucleotides and 537 nucleotides downstream of a stop codon. However, no in-frame ATG codon upstream of the known amino acid sequence was found. To search for the translational start site, the HX 1-1 cDNA (995 bp), containing the most 5′-sequences and a synthetic oligonucleotide from nucleotide 61 to 79, were used to screen a rabbit genomic library. Southern blot analysis of the single positive clone identified a 6.6-kb SphI genomic fragment which hybridized with the 995-bp cDNA fragment. Sequence analysis revealed that 108 bp of an intron were present at the 3′-end of the SphI fragment, which continued upstream in the coding region at nucleotide 790. By utilizing as primer an oligonucleotide corresponding to nucleotide 61–79, sequences were extended at the 5′-end by 291 bp. In this region were several stop codons in all reading frames and no intron/exon boundary consensus sequence (51). Only two ATG codons were found, both in the same reading frame. The first was followed by an in-frame stop codon and the second was located 10 bp upstream from the cDNA 5′-end. The GCCCAATG sequence around the second ATG is in reasonable agreement with the Kozak's consensus sequence (52). Thus, it seems likely that this ATG is the translational initiation codon. The COOH terminus is defined by a TAA codon which is followed by several other stop codons in all reading frames. No polyadenylation signal was found in the 537 bp of the 3′-untranslated region. Sequence data from all the clones establish an open reading frame of 3327 bases (Fig. 2) encoding a protein of 1109 amino acids with a Mr of 124,257. The amino acid sequences of six peptides obtained from purified rabbit skeletal muscle R<sub>c11</sub> subunit, provided by Dr. Philip Cohen (University of Dundee, Dundee, Scotland), were present in the deduced sequences (Fig. 2) with only two mismatches, cysteine residues at positions 26 and 183 for tyrosine and valine, respectively. Hydroxylation analysis by the method of Rao and Argos (53) indicated a region, at the COOH terminus between residues 1063 and 1097, rich in hydrophobic residues and which predicts a transmembrane helix.

 Examination of the nucleotide sequences of all the cDNA clones characterized indicated the existence of two groups, clones HX 13-2, HX 1-2, HX 11-2, and HX 16-1 in one group and clones HX 1-1, HX 11-1, HX 5-1, and HX 15-1 in the
putative cap site was observed also in RNA prepared from rabbit skeletal muscle, a major transcriptional translational start codon ATG was identified (Fig. 3). This start point located at a

extension analysis was carried out in order to map the 5'-end lysine, respectively. The other four differences were silent. Changes in amino acid sequences at residue 311 from threonine to methionine and at residue 413 from asparagine to glutamine were observed. Most likely this discrepancy is due to allelic variations.

Fig. 2. Nucleotide and deduced amino acid sequences of rabbit skeletal muscle RCL subunit. The complete sequence was obtained by combining all the clones shown in Fig. 1. A single underline indicates peptide sequences derived from the RCL subunit polypeptide and a double underline indicates the amino acid residues used for synthesis of the oligonucleotide probes employed in the screening. The dotted line indicates the region rich in hydrophobic residues.

second. The two groups differed in six nucleotides, at positions 703, 744, 1176, 1302, 944, and 1251. The last two caused changes in amino acid sequences at residue 311 from threonine to methionine and at residue 413 from asparagine to lysine, respectively. The other four differences were silent. Most likely this discrepancy is due to allelic variations.

Determination of the Transcriptional Start Site—Primer extension analysis was carried out in order to map the 5'-end of the rabbit RCL subunit transcript. Utilizing total RNA prepared from rabbit skeletal muscle, a major transcriptional start point at a C located 12 nucleotides upstream from the transcriptional start codon ATG was identified (Fig. 3). This putative cap site was observed also in RNA prepared from rabbit diaphragm and cardiac muscle but not from lung (data not shown) in which no homologous RCL subunit appears to be expressed.

Tissue Distribution of the RCL Subunit mRNA and Southern Analysis of Genomic DNA—The tissue distribution and the complexity of the RCL subunit mRNA was investigated by Northern analyses. Total RNA from various rabbit tissues was hybridized with the labeled cDNA inserts from clone HX 1-1 (995 bp) and from clone HX 1-2 (2.95 bp) as probes. A major hybridizing mRNA species at 7.5 kb was observed in heart, skeletal muscle, and lung. A transcript of approximately 5.5 kb was also detected in brain and kidney. The complexity of the RCL mRNA appeared to be higher in skeletal than in cardiac muscle. However, none of the mRNA species was present in brain, kidney, liver, and lung, although staining of the gel with ethidium bromide indicated that
FIG. 3. Mapping of the 5'-end of Rcl subunit mRNA. Primer extension analysis was carried out as described under “Experimental Procedures.” The primer extended DNA fragment obtained from rabbit skeletal muscle mRNA (RSM) and the corresponding sequence of the 6.6-kb SphI genomic DNA fragment are presented. The arrow at the right indicates the position of the major labeled extended product and the arrow at the left the transcriptional start site.

FIG. 4. Northern blot analysis of RGl subunit mRNA. Total RNA (15 µg/lane) prepared from the indicated rabbit tissues was electrophoresed through an agarose gel under denaturing conditions and transferred to a nitrocellulose membrane. Hybridization was carried out as described under “Experimental Procedures.” Numbers indicate the size of molecular markers in kilobases.

FIG. 5. Southern blot analysis of rabbit genomic DNA. Rabbit genomic DNA (20 µg/lane) digested by the indicated restriction endonucleases was electrophoresed in an agarose gel, transferred to nitrocellulose membrane, and probed with the 32P-labeled 995-bp cDNA fragment of clone HX 1-1. Numbers indicate the size, in kilobases, of markers.

Expression of Recombinant Rcl Subunit in E. coli Cells—
The structure of the polypeptide encoded by the composite cDNA was examined by expression in E. coli. Construction of the expression vector G.pET-8c as described under “Experimental Procedures” is illustrated in Fig. 6. E. coli BL21(DE3) cells transfected with the G.pET-8c plasmid were grown and lysed as described under “Experimental Procedures.” Analysis of the cell extracts by Western immunoblotting indicated the presence of three major immunoreactive polypeptides, one of which had an apparent Mr of approximately 160,000, similar to that observed in rabbit skeletal muscle extracts (Fig. 7). Similar results were also obtained when the E. coli cells were directly treated with 0.5% SDS at 100 °C for 5 min, before Western analysis (not shown). The amount of immunoreactive material increased with increasing concentrations of IPTG from 0.5 to 1 mM (Fig. 7, panel B: lanes 2, 3, 5, and 6) and was absent in extracts from untransfected cells (lanes 7-9). The slight amount of polypeptides detected in extracts of G.pET-8c-harboring cells not induced by IPTG is attributed to the basal T7 RNA polymerase activity. Increasing time (data not shown) and induction by IPTG appeared to generate proportionally more of the lower molecular weight species. When the cells were lysed in buffer without 1% Triton X-
The orientation of ampicillin-resistant gene sequences. The particulate fraction (data not shown). Expression of immunoreactive polypeptides. The procedure for construction of the expression vector is described under "Experimental Procedures." The sites for restriction enzymes are indicated: \( \text{BamH} \), \( \text{BglII} \), \( \text{EcoRI} \), \( \text{NcoI} \), \( \text{RsaI} \), and \( \text{SphI} \). The filled area represents the \( \text{pET-8c} \) vector, and the dashed portion shows the \( \Phi 10 \) promoter and the Shine/Dalgarno (\( S/D \)) sequences. The open area indicates the assembled \( \text{R}_{\text{c}} \) subunit cDNA. The orientation of ampicillin-resistant gene (\( \text{bla} \)) and of the replication origin (\( \text{ori} \)) are shown by arrows.

100, most of the immunoreactive material was associated with the particulate fraction (data not shown). Expression of \( \text{R}_{\text{c}} \) subunit cDNA was achieved only when freshly transfected cells were used. Transfected \( \text{BL}21(\text{DE}3) \) cells stored at \(-80 ^\circ \text{C} \) in 15% glycerol or on agar plates significantly lost their ability to express immunoreactive polypeptides.

**DISCUSSION**

We report the isolation and characterization of cDNAs encoding the regulatory (\( \text{R}_{\text{c}} \)) subunit of the rabbit skeletal muscle glycogen-associated type 1 protein phosphatase. Sequencing of 23 cDNA clones failed to provide a translational ATG start codon, which was obtained from the isolation of a rabbit genomic clone. Primer extension analysis indicated that the 5' -untranslated region is very short, 12 nucleotides. The procedure of Gubler and Hoffman (54), which can lead to the loss of the first 20–30 nucleotides, was employed in the construction of our cDNA libraries and could explain the absence of the initiation codon in the cDNA clones isolated. Combined overlapping nucleotide sequences established an open reading frame coding for 1109 amino acids with a \( M_{r} \), 124,257. Immunoblot analysis of rabbit skeletal muscle extracts had estimated the apparent molecular weight of \( \text{R}_{\text{c}} \) subunit to be approximately 160,000 (28, 29), which is clearly larger than that deduced from the nucleotide sequence. Two explanations could be advanced for this discrepancy: the first is that we did not have the complete coding region and the second that the rabbit skeletal muscle \( \text{R}_{\text{c}} \) subunit is post-translationally modified, leading to a lower mobility on SDS-PAGE. However, expression of the cDNA in \( \text{E. coli} \) demonstrates that the deduced primary structure is complete, since a polypeptide with the same electrophoretic mobility as that present in skeletal muscle extract could be detected. Since post-translational modification in \( \text{E. coli} \) is unlikely, the larger size estimated by gel electrophoresis must be explained by an intrinsic property of the protein. Interestingly, two other regulatory proteins of type 1 phosphatase, inhibitor-1 and inhibitor-2, have also been shown to migrate anomalously on SDS-PAGE, with apparent molecular weights of 26,000 instead of 19,000 (55, 56) and 31,000 instead of 23,000 (28, 57, 58), respectively. In all three instances the polypeptides have been shown to be highly asymmetric as evidenced by their large Stokes radius and small sedimentation constants (20, 55–58). In all cases, the size estimated by amino acid sequence is approximately 70% of that determined by SDS-PAGE. However, despite sharing these properties, the three proteins show no resemblance in sequence.

The lower molecular weight polypeptides, 58 and 46 kDa (Fig. 7), detected in extracts of \( \text{E. coli} \) transfected with \( \text{R}_{\text{c}} \) subunit cDNA, confirm the extreme sensitivity of the polypeptide to proteolysis. The observation that the same species are also present when the cells are disrupted directly by 100 °C heat treatment in the presence of SDS (data not shown) suggests that degradation might be occurring inside the cell and not during processing of the extracts. Such an occurrence is not unusual and has been reported for other mammalian polypeptides expressed in bacteria (59, 60). \( \text{R}_{\text{c}} \) subunit degradation products of similar molecular weight have been observed in preparations of rabbit skeletal muscle glycogen-associated phosphatase (27, 61), which might indicate that specific regions of the polypeptide are especially sensitive to...
proteolytic cleavage. We can exclude that the 58- and 46-kDa forms are generated by initiation of translation at a downstream ATG, since the antibodies used for the detection were raised to the region corresponding to residues 37 to 56 and no other ATGs are present in this NH2-terminal region.

Recently Hubbard et al. (22) reported that a sarcoplasmic reticulum-associated phosphatase contains a polypeptide similar, if not identical, to the RcG subunit. Hydrophathy analysis of the deduced amino acid sequence reported here indicated a potential transmembrane region between residues 1063 and 1097 (Fig. 2), which could be responsible for anchoring the protein to the membrane. Thus, the same regulatory subunit might function to target the phosphatase to membranes and glycogen. Several lines of evidence argue against the existence of distinct tissue isozymes. The low molecular weight polypeptides observed in glycogen-associated phosphatase purified from rabbit skeletal muscle are not always detected by Western immunoblotting analysis (Fig. 7) and Northern hybridization, utilizing 3,850 bp of cDNA sequence, indicated one major mRNA species. The minor differences observed in the two groups of cDNA clones, four silent nucleotide substitutions and two changing the amino acid residues could be explained by allelic variations. Southern analysis also suggested a single gene. Studies in progress, with mutant protein in which the hydrophobic region has been deleted, should prove useful in addressing the question of how the same polypeptide might be directed to different cellular compartments.

Analysis of the tissue distribution of the RcG subunit mRNA (Fig. 4) supports previous observations by Western immunoblot (28), which indicated that the polypeptide is specifically expressed in skeletal and cardiac muscle, but not in other tissues examined. This also suggests that the polypeptide responsible for targeting the phosphatase to glycogen in liver is not homologous to the muscle form, although their molecular weights appear to be very similar and both interact with the highly conserved catalytic subunit (62).

The rabbit skeletal muscle glycogen-associated phosphatase undergoes in vivo and in vitro phosphorylation at several sites all of which are located near the NH2 terminus. The cAMP-dependent protein kinase sites are at residues 48 and 67, respectively, and the glycogen synthase kinase-3 sites at positions 40 and 44. Other potential recognition sites for cAMP-dependent protein kinase are threonine 498 and 978 and the glycogen synthase kinase-3 sites at positions 40 and 44. Other potential recognition sites for CAMP-dependent protein kinase might have been missed. Threonine 978, in the motif -Arg-Arg-Val-Thr-, is an especially strong candidate. Phosphorylation of this residue, similarly to serine 48, could form a recognition site for glycogen synthase kinase-3 (31).

Search of the Swiss protein (release 13) and the EMBL (release 21) data bases with PCGENE utilizing the FASTN and FASTP programs (63) revealed no significant homology between the RcG subunit and other known sequences. However, a search of the protein data base assembled by Dr. Mark Goebi, at Indiana University, indicated significant homology with the product of the yeast gene, GAC1, isolated by Dr. Kelly Tatchell at North Carolina State University.4 Over a segment of 144 residues, the identity is 27% and the homology 38% if conservative replacements are taken into account.5 Utilizing the algorithm of Lipman and Pearson (63), the optimal alignment score between RcG and GAC1 amino acid sequence is 13 standard deviations over the mean of the optimal score of 100 random shufflings of the GAC1 sequence. It is of significance that the similarity lies within the 40-kDa NH2-terminal portion of the RcG protein, which is able to interact with glycogen and with the catalytic subunit of type 1 phosphatase (61). The GAC1 protein appears to be involved in activation of glycogen synthase and glycogen accumulation. Both of these functions are consistent with the GAC1 gene product being the yeast homologue of the RcG subunit. Gene replacement in yeast should allow us to address this question. In addition site specific and deletion mutagenesis will provide a powerful tool to elucidate the physiological role and regulation of the glycogen-associated protein phosphatase.

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5 The following groupings of amino acids were considered homologous: Val-ILE-Leu; Phe-Tyr; Ala-Ser-Thr; Asp-Glu; Asn-Gln; Arg-Lys.

4 K. Tatchell, personal communication.

3 K. M. Swiderek and A. A. DePaoli-Roach, unpublished results.
