Parafusin, an Exocytic-sensitive Phosphoprotein, Is the Primary Acceptor for the Glucosylphosphotransferase in Paramecium tetraurelia and Rat Liver

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Abstract. Parafusin, the major protein in Paramecium tetraurelia to undergo dephosphorylation in response to secretory stimuli, appears to be the primary acceptor for the glucosylphosphotransferase in this species based on five independent criteria: identical molecular size of 63 kD; identical isoelectric points in the phosphorylated state of pH 5.8 and 6.2; identical behavior in reverse-phase chromatography; immunological cross-reactivity with an affinity-purified anti-parafusin antibody; the presence of a phosphorylated sugar after acid hydrolysis. It appears likely that the dephosphorylation observed with secretion reflects the removal of αGlc-1-P from parafusin's oligosaccharides and is consistent, therefore, with a regulatory role for this cytoplasmic glycosylation event. The glucosylphosphotransferase acceptor in rat liver is also immunoprecipitated by the anti-parafusin antibody and is very similar in physical characteristics to the paramecium protein. This conservation suggests a role for parafusin in mammalian exocytosis as well, at a step common to both the regulated and constitutive secretory pathways.

INTRODUCTION. Paramecium tetraurelia is an attractive model system for the study of regulated secretion since massive and synchronous exocytosis can be initiated with an appropriate stimulus. In paramecium, parafusin, a cytoplasmic phosphoprotein of 63 kD, is the major protein to undergo dephosphorylation during exocytosis (Gilligan and Satir, 1982; Zieseniss and Plattner, 1985). Functionally, parafusin may be involved in regulation of a membrane fusion step after the required influx of extracellular Ca^{2+} has taken place (Gilligan and Satir, 1983). Parafusin has been purified and a polyclonal antibody against it produced (Murtaugh et al., 1987). It has three isoforms, with isoelectric points of 5.8, 6.2, and 6.3, the former two being phosphorylated and cytosolic. Parafusin has been shown to be immunologically cross-reactive with proteins from a variety of sources including yeast, cockroaches, toad bladder, rat liver, and other tissues, and bovine and human brain (Satir et al., 1988, 1989).

Here, we report that paramecium parafusin is the principal acceptor for the UDP-glucose:glycoprotein glucose-1-phosphotransferase (Glc-phosphotransferase), an enzyme that catalyzes the transfer of αGlc-1-P from UDP-Glc to mannose residues on acceptor glycoproteins (Koro and Marchase, 1982; Hiller et al., 1987). The Glc-phosphotransferase and an acceptor of 62-64 kD have, like parafusin, been shown to be present in a wide variety of eukaryotes, including yeast, spinach, sea urchin, and a spectrum of mammalian tissues (unpublished results).

Our collaborative investigation exploring a possible relationship between parafusin and the Glc-phosphotransferase acceptor was begun because of similarities in their apparent size, in their subcellular distribution, and in their ubiquity.
in eukaryotes. The evidence that in paramecium these phosphoproteins are one and the same includes identity of apparent molecular weights in SDS-PAGE and of pI's in analytical isoelectric focusing, and the finding that the acceptor labeled by the Glc-phosphotransferase can be immunoprecipitated with an affinity-purified anti-parafusin antibody. In addition, acid hydrolyses of paramecium parafusin labeled in vivo with \(^{32}P\), yields a labeled sugar phosphate. The Glc-phosphotransferase acceptor in rat liver is similar in these physical characteristics to paramecium parafusin, and can also be immunoprecipitated by the paramecium anti-parafusin antibody.

These findings suggest that parafusin, which is highly conserved among eukaryotes, is a novel cytosolic phosphoglycoprotein and that reversible phosphoglycosylation of parafusin could play an important role in signal transduction during secretion in paramecium and perhaps in mammalian cells as well.

### Materials and Methods

#### Culturing, Harvesting, and In Vivo \(^{32}P\), Labeling of Paramecium

Wild-type *Paramecium tetraurelia* were grown to early stationary phase (≏20,000 cells/ml) in axenic medium (Soldo et al., 1966) and then harvested by centrifugation in an IEC clinical centrifuge (500 g, 5 min), washed in phosphate-buffered saline (PBS) containing 1 mM CaCl\(_2\), 20 mM MgCl\(_2\), 1 mM KCl, pH 7.0, and concentrated 100-200-fold. Cells were labeled in vivo with \(^{32}P\), as described in Gilligan and Satir (1982).

#### Production of an Antibody to Parafusin

Preparation of paramecium parafusin and affinity-purified antibody to parafusin was performed as described in Murtaugh et al. (1987). Transfer to nitrocellulose and immunoblotting with this antibody were carried out as described in Satir et al. (1990).

#### Glc-Phosphotransferase Assays

Paramecium cultures were grown to early stationary phase (≏2000 cells/ml) in axenic medium (Soldo et al., 1966) and then centrifuged in an IEC clinical centrifuge (500 g, 5 min), washed in phosphate-buffered saline (PBS) containing 1 mM CaCl\(_2\), 20 mM MgCl\(_2\), 1 mM KCl, pH 7.0, and concentrated 100-200-fold. Cells were labeled in vivo with \(^{32}P\), as described in Gilligan and Satir (1982).

#### Acid Hydrolyses of \(^{32}P\)-Labeled Phosphoproteins

Phosphoproteins labeled via incubation with \(^{32}P\)UDP-Glc were separated from precursor by chromatography in H\(_2\)O over Sephadex G-75, lyophilized, and hydrolyzed in 1 N HCl at 100°C for 1 h. The hydrolyzed products were diluted with H\(_2\)O, lyophilized, and subjected to chromatography in 0.05 N HCl over Dowex 50W (H\(^{+}\) form) as described by Schaffer (1967). In vivo \(^{32}P\)-labeled parafusin was immunoprecipitated as described above, and aliquots were subjected to either hydrolytic conditions or to 2 N HCl, 100°C, 8 h. The samples were then diluted, lyophilized, and chromatographed as described above. \(^{3}H\)Man-6-P was prepared from \(^{3}H\)Man with hexokinase and ATP. This was used as a standard for calibrating the Dowex 50 W column, along with \(^{32}P\), phosphoserine, phosphothreonine and phosphotyrosine. Phosphoamino acid standards were detected using a ninhydrin reagent (Marchase et al., 1990).

### Results

#### The Glc-Phosphotransferase Adds \(^{32}P\) to Mannose Residues on a 63-kD Acceptor in Paramecium

To determine if paramecium possessed the Glc-phosphotransferase and, if so, to determine the molecular masses of its acceptors, homogenates prepared from the cells were incubated with \(^{32}P\)UDP-Glc, subjected to SDS-PAGE, and autoradiographed. An acceptor of 63 kD was found to be the primary band labeled in the autoradiograph (Fig. 1, lane 1), nearly identical in molecular size to the primary acceptor for the Glc-phosphotransferase previously reported in rat liver (Srisommap et al., 1988). These incubations were performed in the presence of 1 mM unlabeled ATP and P\(_i\) to dilute nonspecific kinase utilization of contaminants and breakdown products. In addition, 100 \(\mu\)M \(^{32}P\)UDP-Glc was included to dilute any possible labeling of glucokinase, phosphofructokinase, and glucose-6-phosphatase dehydrogenase. The radioactive labeling appeared to be due to direct incorporation from UDP-Glc, since additional 100 \(\mu\)M unlabeled UDP-Glc was found to effectively
Figure 1. The Glc-phosphotransferase labels a 63-kD acceptor in paramecium homogenates. Lane 1, autoradiograph after SDS-PAGE of proteins from paramecium homogenates after incubation for 30 min with 5 μCi [β³²P]UDP-Glc, 1 mM ATP, 1 mM Pi, and 100 μM αGlc-1-P. Lane 2, autoradiograph following identical protocols except that an additional 100 μM unlabeled UDP-Glc was present during the incubation. Lane 3, autoradiograph following protocol described for lane 1 except that αGlc-1-P concentration was 200 μM.

dilute the observed labeling (Fig. 1, lane 2), while increasing the αGlc-1-P concentration to 200 μM caused only limited loss of label (lane 3) due to a slight inhibitory effect on the transferase (unpublished observation).

Further evidence that the observed label was due to the action of the Glc-phosphotransferase came from analyses of the labeled product of acid hydrolyses (1 N HCl, 100°C, 1 h) of the phosphoprotein, in which terminal Glc residues would be cleaved at the phosphodiester bond while the underlying

Figure 2. Acid hydrolysis of the paramecium protein labeled with [β³²P]UDP-Glc yields a phosphorylated sugar and no evidence of phosphorylated amino acids. Column chromatography over Dowex 50W (H⁺ form) in 0.05 N HCl following acid hydrolysis of paramecium homogenates labeled with [β³²P]UDP-Glc yielded a ³²P-labeled moiety that co-chromatographed with [³H]Man-6-P and not with phosphoserine, phosphothreonine, or phosphotyrosine. Arrows indicate elution peaks of standards. Phosphothreonine and phosphotyrosine eluted near phosphoserine.

Figure 3. Enzymatic conversion of the ³²P-labeled product of the acid hydrolysis to 6-phosphogluconate suggests that it is [³²P]Man-6-P. The ³²P-labeled product of acid hydrolysis was found to comigrate with [³H]Man-6-P in paper electrophoresis carried out in H₂O/pyridine/acetic acid (2:1). After treatment with glucose-6-phosphate dehydrogenase, phosphomannose isomerase, and phosphoglucone isomerase (Gawehn, 1974), the unknown (●) was partially converted to 6-phosphogluconate (6PG), consistent with it initially being [³²P]Man-6-P.

Figure 4. The Glc-phosphotransferase acceptor and parafusin are identical in apparent molecular mass in both paramecium and isolated rat hepatocytes. (A) Autoradiograph after SDS-PAGE and transfer to nitrocellulose. Lane 1, homogenized paramecium labeled with [³⁵S]UDP-Glc. Lane 2, high speed supernatant (S2) after in vivo labeling of paramecium with ³²P. Lane 3, homogenized hepatocytes labeled with [³⁵S]UDP-Glc. (B) Immunoblot using the paramecium anti-parafusin antibody of the same samples shown in A. Note in each case the samples show cross-reactivity at M₅, 63,000 (arrow).
pooled and incubated with glucose-6-phosphate dehydrogenase, phosphomannose isomerase, and phosphoglucone isomerase in the presence of NADP⁺ (Gawehn, 1974). The enzyme-containing and control aliquots were then subjected to high voltage paper electrophoresis. When none of the enzymes was present, the ³²P unknown was found to co-migrate with the [³H]Man-6-P standard. In the enzyme-treated sample, the ³²P-labeled unknown was partially converted to 6-phosphogluconate (Fig. 3). This result thus suggests that the underlying sugar on the acceptor for the Glc-phosphotransferase is identical to what was found in chick neural retina (Koro and Marchase, 1982) and rat liver (Marchase et al., 1990).

The Principal Acceptor for the Glc-Phosphotransferase in Paramecium Is Identical to Parafusin in Several of Its Physical Properties

To determine more precisely how the principal Glc-phosphotransferase acceptor in paramecium compared in molecular size to parafusin, homogenates prepared from the cells were incubated with the [³⁵S]UDP-Glc labeled phosphorothioate analogue of UDP-Glc ([³⁵S]UDP-Glc), subjected to SDS-PAGE, and blotted to nitrocellulose (Fig. 4 A, lane 1). Parafusin, one of the most heavily phosphorylated proteins found after in vivo labeling of paramecium with ³²P, was electrophoresed in an adjacent lane (Fig. 4 A, lane 2). An apparent correspondence in molecular size was found, and more precisely confirmed by comparing the autoradiographs to Western blots (Fig. 4 B, lanes 1 and 2) stained using an affinity purified antibody to parafusin (Murtaugh et al., 1987). The autoradiograph of the band labeled with [³⁵S]-UDP-Glc corresponded precisely to the band visualized in the Western blot of the same lane.
For comparison, homogenate prepared from rat hepatocytes was also incubated with [β³⁵S]UDP-Glc and electrophoresed (Fig. 4 A, lane 3). A primary acceptor of nearly the same molecular size is seen (Srisomsap et al., 1988), as well as smaller peptides attributable to proteolysis (unpublished observations). Fig. 4 B, lane 3 demonstrates cross-reactivity of the anti-parafusin antibody with a liver protein of this apparent molecular mass (Satir et al., 1989).

Analytical isoelectric focusing of parafusin and the acceptor for the Glc-phosphotransferase in paramecium also suggested identity. Parafusin was labeled in vivo with ³²P, and then purified by immunoprecipitation with the affinity-purified antibody. The ³²P-labeled sample was then compared by analytical isoelectric focusing and autoradiography with a paramecium homogenate labeled in vitro through incubation with [β³⁵S]UDP-Glc. As is shown in Fig. 5 A, lane 1, the UDP-Glc-labeled sample produced predominantly two isoforms of pI 5.8 and 6.2, as did the immunoprecipitated in vivo ³²P-labeled parafusin, although the more acidic form is only barely visible in the photograph (Fig. 5 A, lane 2). This latter result confirms the finding reported by Murthaugh et al. (1987) of two phosphorylated forms of parafusin with precisely these pls. In addition, the liver and paramecium proteins labeled in vitro with [β³⁵S]UDP-Glc are strikingly similar in isoforms (Fig. 5 B, lanes 1 and 2).

The Glc-phosphotransferase acceptor labeled through incubation in paramecium homogenates with [β³⁵S]UDP-Glc was also compared with in vivo ³²P-labeled parafusin on C-4 reverse-phase chromatography, in which separation is based predominantly on hydrophobicity. Their elution profiles (Fig. 6, B and C) both peaked at 25 min (corresponding to 58% acetonitrile), again suggesting identity. Similarity to the [β³⁵S]UDP-Glc-labeled protein labeled in rat liver is again seen (Fig. 6 D), although the liver protein displayed a slightly delayed retention time.

**Anti-Parafusin Antibody Immunoprecipitates the Acceptor Labeled by the Glc-Phosphotransferase in Both Paramecium and Rat Liver**

An additional test for the identity of parafusin and the acceptor for Glc-phosphotransferase in paramecium was performed using the criterion of immunoprecipitation with the affinity-purified anti-parafusin antibody (Murthaugh et al., 1987). Fig. 7 shows an autoradiograph of the results from an immunoprecipitation of a [β³⁵S]UDP-Glc-labeled paramecium homogenate. The first two lanes compare the supernatant (S) after precipitation with the anti-parafusin antibody (Ab+) to the supernatant from the control (Ab−). Radioactivity was completely removed from the supernatant exposed to anti-parafusin. The final two lanes compare the SDS-solubilized pellets (P) resulting from these precipitations. Radioactivity at 63 kD is greatly enhanced in the pellet exposed to antibody. Thus, both by the three independent physical characteristics described above and by immunocross-reactivity, the acceptor labeled by the Glc-phosphotransferase in paramecium and the phosphoprotein previously identified as parafusin are indistinguishable.

Fig. 8 is an autoradiograph of an SDS-PAGE showing an immunoprecipitation experiment after labeling of rat hepatocyte homogenate with [β³⁵S]UDP-Glc. The labeled hepatocyte homogenate (Fig. 8, lane 1) was centrifuged to remove cell debris. As expected, the resulting supernatant (Fig. 8, lane 2) was somewhat enriched in labeled protein, but some proteolysis was also evident. Following a mock immunoprecipitation (Ab−), all the 63-kD protein remained in the supernatant (Fig. 8, lane 3). The corresponding pellet (Fig. 8, lane 8) contained >4% of total counts, approximately background level. In contrast, after immunoprecipitation with the anti-paramecium parafusin antiserum (Ab+), ~40% of the labeled 63-kD protein from the hepatocytes was immunoprecipitated (Fig. 8, lanes 6 and 7), the remaining label being found in the supernatants (lanes 4 and 5).

**Acid Hydrolyses of Parafusin Labeled In Vivo with ³²P**

Paramecium parafusin was labeled in vivo with ³²P, and immunoprecipitated with the affinity-purified anti-parafusin antibody. This preparation was then split into halves and acid hydrolyzed under two conditions, one optimal for recovery of phosphoserine and the other for Man-6-P. They were then subjected to chromatography over Dowex 50W (H+ form), as described above. After hydrolysis under the milder conditions (Fig. 9), 30% of the counts were found to migrate with an internal [¹H]Man-6-P standard, the remaining 70% being ³²P. When the more stringent hydrolytic conditions were used, nearly all the radioactivity coeluted from the Dowex column with ³²P (data not shown). There was thus no evidence for the presence of a phosphorylated amino acid under either of these hydrolytic conditions.

**Discussion**

We have established that, like other eukaryotic cells, paramecium possesses a Glc-phosphotransferase that adds α-Glc-1-P to mannose residues on a cytosolic 63-kD acceptor. Five independent criteria support the conclusion that parafusin, a protein that evidently is associated with membrane fusion during exocytosis in paramecium (Satir et al., 1989), is this...
Figure 8. The Glc-phosphotransferase acceptor from rat hepatocytes is immunoprecipitated by anti-parafusin. Autoradiograph of SDS-PAGE supernatants (S) and pellets (P) obtained from rat hepatocyte homogenates incubated with [β³²S]UDP-Glc and subjected to immunoprecipitation with anti-parafusin antibody. Lane 1 shows initial homogenate. Lane 2 shows the low speed supernatant used in the immunoprecipitation. Some proteolysis of the 63-kD protein is evident. Lane 3 (Ab−) shows 1% of the supernatant after a mock (no primary antibody) immunoprecipitation, and lane 8 shows 10% of the corresponding pellet. Lanes 4 and 5 show 1% of the supernatant after immunoprecipitation with the anti-parafusin antibody (Ab+). Lanes 6 and 7 show 10% of the corresponding pellets. In lanes 4 and 6, the sample was boiled in 2% SDS before exposure to the antibody; in lanes 5 and 7 the sample was not boiled.

Figure 9. Acid hydrolysis of immunoprecipitated parafusin after in vivo labeling of paramecium with 
³²P, yields 
³²P, and 
³²P but no phosphorylated amino acids, as assessed by column chromatography over Dowex 50W (H⁺ form) in 0.05 N HCl.

The possible involvement of parafusin in exocytosis was first inferred in experiments with axenic cultures of paramecium, which take up 
³²P, and phosphorylate a small number of polypeptides. It was found that the most heavily labeled polypeptide was a minor component, parafusin. This polypeptide was linked to the process of membrane fusion and exocytosis in these cells in a series of in vivo studies (Gilligan and Satir, 1982, 1983) that showed that when prelabeled wild-type cells were stimulated to secrete the labeled parafusin was dramatically dephosphorylated.

In the present study in vivo 
³²P incorporation into parafusin, after acid hydrolysis, yields a phosphorylated sugar. An earlier report suggested the presence of phosphorylated serine in paramecium parafusin (Satir and Murtaugh, 1988). The possibility exists that both phosphoserine and a phosphorylated oligosaccharide are present in parafusin and that conditions in its preparation are crucial for maintaining both. Phosphorylated serine may, for instance, be affected by phosphatases during preparation, as was found during isolation of parafusin (Murtaugh et al., 1987). Alternatively, phosphoglucomutase has a molecular size of 62 kD and contains phosphoserine (Ray et al., 1983). Since the initial hydrolyses were performed on labeled material enriched solely by SDS-PAGE, its presence could have influenced the previous result. In any case, since nearly a complete loss of phosphate from parafusin is seen after stimulation of exocytosis (Gilligan and Satir, 1982, 1983), removal of αGlc-1-P from parafusin seems likely. However, this point still remains to be clarified.

If, in fact, the dephosphorylation of parafusin that occurs as exocytosis is initiated is due to the removal of αGlc-1-P from its oligosaccharide, this would suggest that carbohydrates on cytoplasmic glycoproteins may be cyclically added and/or removed rapidly in response to external stimuli. In this case, such a removal could be catalyzed by an αGlc-1-P acceptor for the Glc-phosphotransferase. These criteria include: (a) identity in apparent molecular mass between parafusin and the Glc-phosphotransferase acceptor as determined by SDS-PAGE; (b) identity in hydrophobicity as assessed by their behavior in C4 reverse-phase chromatography; (c) identity in isoelectric points as determined by analytical isoelectric focusing; and more conclusively, (d) an affinity-purified antibody prepared against parafusin recognized the Glc-phosphotransferase acceptor in paramecium, and (e) in vivo 
³²P-labeled immunoprecipitated parafusin was shown to contain labeled phosphosugar after acid hydrolysis. Until complete amino acid sequence analyses are obtained from both proteins, we tentatively conclude that the proteins are identical.

The acceptor for the Glc-phosphotransferase in rat liver and paramecium parafusin have also been found to be virtually identical with respect to molecular size, isoelectric profiles, and reverse-phase elution characteristics. Furthermore, the liver acceptor is immunoprecipitated by antibody prepared against parafusin isolated from paramecium. These results appear to reflect a remarkable conservation of parafusin (the Glc-phosphotransferase acceptor) in two evolutionarily widely divergent species. The ubiquity of a cytoplasmic phosphoglycoprotein of ~63 kD, as assessed by cross-reactivity with the antibody specific for paramecium parafusin (Satir et al., 1989) and by the presence of an acceptor of that size for the Glc-phosphotransferase across all eukaryotes examined including yeast, invertebrates, and a variety of mammalian tissues, suggests an even broader evolutionary and possibly functional conservation of the molecule.

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phosphodiesterase similar to that described in rat liver (Srisomsap et al., 1989), which is apparently specific for the Glc-P-Man diester. This cycling in oligosaccharide structure may reflect but one example of a group of regulated posttransla-
tional modifications in which sugars are used to modulate a protein’s behavior. The majority of glycosylation events occur as newly synthesized proteins mature while moving vectorially through the ER and Golgi apparatus. However, cytoplasmic glycosylation presents different possibilities. A cytoplasmic glyco-
protein could be repeatedly exposed to glycosyltransferases and antagonistic glycosidases, free from compartmentaliza-
tion constraints that discourage cyclical glycoprotein modifications within the ER and Golgi apparatus. The data pre-
sented here suggest that such a cycle could be involved in exocytosis. The presence of parafusin in liver may imply a role for the molecule at a step common to both constitutive and regulated secretion.

Cytoplasmic glycosylation may, therefore, function in a very different manner from glycosylation within the ER and Golgi apparatus. There are now several other examples of cytosolic glycosylation events (Hart et al., 1989), the best studied of which gives rise to O-linked GlicNAc’s present, for instance, on nuclear pore (Davis and Blobel, 1987) and chromatin (Kelly and Hart, 1989) proteins. Any of these events could cyclically be exposed to antagonistic glycosidases and transferases within the cytosolic compartment. One rather special case is glycojenin, the core protein on which glycogen is synthesized and degraded in the cytosol in a regulated manner (Whelan, 1986). The data presented here may thus reflect but one example of a group of regulated posttransla-
tional modifications in which sugars are used to modulate a cytosolic protein’s behavior.

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References

Amy, C., and N. Kirschner. 1980. Phosphorylation of adrenal medulla cell pro-
teins in conjunction with stimulation of catecholamine secretion. J. Neuro-
chem. 36:847-855.
Burgoyne, R. D. 1984. Mechanisms of secretion from adrenal chromaffin cells. Biochim. Biophys. Acta. 779:201-216.
Cohen, P. 1989. The structure and function of protein phosphatases. Annu. Rev. Biochem. 58:453-508.
Davis, L. I., and G. Blobel. 1987. Nuclear pore complex contains a family of glycoproteins that includes p62: glycosylation through a previously un-
identified cellular pathway. Proc. Natl. Acad. Sci. USA. 84:7552-7556.
Dunkley, P. R., C. M. Baker, and P. J. Robinson. 1986. Depolarization-
dependent protein phosphorylation in rat cortical synaptosomes: character-
ization of active protein kinases by phosphopeptide analysis of substrates. J. Neurochem. 46:1692-1703.
Gaweith, K. 1974. D-Mannose and D-mannose-6-phosphate. In Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, Inc., New York. 1263-1267.
Gilligan, D. M., and B. H. Satir. 1982. Protein phosphorylation/dephosphory-
lation and stimulus-secretion coupling in wild type and mutant Paramaecium. J. Biol. Chem. 257:13903-13906.
Gilligan, D. M., and B. H. Satir. 1983. Stimulation and inhibition of secretion in paramecium: role of divalent cations. J. Cell Biol. 97:224-234.
Greengard, P. 1987. Neuronal phosphoproteins: mediators of signal transduc-
tion. Mol. Neurobiol. 1:81-119.
Gutierrez, L. M., J. J. Ballesta, and M. J. Hidalgo. 1988. A two-dimensional electrophoresis study of phosphorylation and dephosphorylation of chro-
matin cell proteins in response to a secretory stimulus. J. Neurochem. 51:1023-1030.
Hart, G. W., R. S. Haltiwanger, G. D. Holt, and W. G. Kelly. 1989. Glycosy-
lation in the nucleus and cytoplasm. Annu. Rev. Biochem. 58:841-874.
Hiller, A. M., L. A. Koro, and R. B. Marchase. 1987. Glucose-l-phospho-
transferase and GlicNAc-l-phosphotransferase have distinct acceptor spe-
cificities. J. Biol. Chem. 262:4377-4381.
Kelly, W. G., and G. W. Hart. 1989. Glycosylation of chromosomal proteins: localization of O-linked N-acetylgalactosamine in Drosophila chromatin. Cell. 57:243-251.
Kornfeld, S. 1987. Trafficking of lysosomal enzymes. FASEB (Fed. Am. Soc. Bio-
chem.) J. 1:462-468.
Korl, L. A., and R. B. Marchase. 1982. A UDP-glucose:glycprotein glucose-
1-phosphotransferase in embryonic chicken neural retina. Cell. 31:739-748.
Lee, S. A., and R. W. Holz. 1986. Protein phosphorylation and secretion in digiotoxin-permeabilized adrenal chromaffin cells. J. Biol. Chem. 261:17089-17098.
Marchase, R. B., A. M. Miller, A. A. Rivera, and J. M. Cook. 1987. The (Br35S) phosphorothioate analogue of UDP-Glc is efficiently utilized by the glucose phosphotransferase and it resistant to hydrolytic degradation. Bio-
chem. Biophys. Acta. 1:157-162.
Marchase, R. B., K. L. Richardson, C. Srisomsap, R. R. Drake, and B. E. 
Haley. 1990. Resolution of phosphoglucomutase and the 62kDa receptor for the glucosylphosphotransferase. Arch. Biochem. Biophys. 280:122-129.
Murtagh, T. J., D. M. Gilligan, and B. H. Satir. 1987. Purification of actin and actin production of an antibody against a 63,000 M, stimulus-sensitive phos-
phoprotein in Paramecium. J. Biol. Chem. 262:15734-15739.
Ray, W. J., Jr., M. A. Hermodson, J. M. Pavathilingam, and W. C. Moloney. 1983. The complete amino acid sequence of rabbit muscle phosphogluco-
mutase. J. Biol. Chem. 258:9166-9174.
Satir, B. H. 1989. Signal transduction events associated with exocytosis in cili-
ates. J. Protozool. 36:382-389.
Satir, B. H., and T. J. Murtagh. 1989. Phosphorylation events in regulation of exocytosis. In Molecular Mechanisms of Membrane Fusion. S. Okhi, D. Doyle, T. D. Flanagan, S. W. Hui, and E. Mayhew, editors. Plenum Publish-
ing Corp., New York. 513-520.
Satir, B. H., G. Busch, A. Vuoso, and T. J. Murtagh. 1988. Aspects of signal 
transduction in stimulus exocytosis-coupling in paramecium. J. Cell Bio-
chem. 36:429-443.
Satir, B. H., T. Hamasaki, M. Reichman, and T. J. Murtagh. 1989. Species 
distribution of a phosphorylation (parafusin) involved in exocytosis. Proc. 
Nat. Acad. Sci. USA. 86:930-932.
Schaffer, N. K. 1967. Analysis for O-phosphorylserine. Biochim. Biophys. 
Acta. 130:139-147.
Srisomsap, C., K. L. Richardson, J. C. Jay, and R. B. Marchase. 1988. Local-
ization of the glucose phosphotransferase to a cytoplasmically accessible site on intracellular membranes. J. Biol. Chem. 263:17792-17797.
Srisomsap, C., K. L. Richardson, J. C. Jay, and R. B. Marchase. 1989. An 
aglucose-l-phosphate phosphodiesterase is present in rat liver cytosol. J. 
Biol. Chem. 264:20540-20546.
Wang, J. K. T., S. I. Walaas, and P. Greengard. 1982. Protein phosphoryla-
tion in nerve terminals: comparison of calcium/calmodulin-dependent and cal-
cium/diacylglycerol-dependent systems. J. Neurosci. 5:136-140.
Wu, W. C.-S., S. I. Walaas, A. C. Naim, and P. Greengard. 1982. Calcium 
phospholipid regulates phosphorylation of M, 87k substrate protein in brain 
synaptosomes. Proc. Natl. Acad. Sci. USA. 79:5249-5255.
Zieseniss, E., and H. Plattner. 1985. Synchronous exocytosis in paramecium 
cells involves very rapid (<1 s), reversible dephosphorylation of a 65kD 
phosphoprotein in exocytosis-competent strain. J. Cell Biol. 101:2028-
2035.

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