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# An 83 kDa O-GlcNAc–Glycoprotein Is Found in the Axoplasm and Nucleus of *Aplysia* Neurons

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**Glycoproteins containing O-linked *N*-acetylglucosamine (O-GlcNAc) are present in axons of *Aplysia* neurons (Gabel et al., 1989) and among transcription factors and other proteins in the nucleus of eukaryotic cells (Jackson and Tjian, 1988). A recently discovered pathway in neurons transports proteins through the axon and then into the nucleus (Ambron et al., 1992). If any of the axonal O-GlcNAc glycoproteins use this pathway, then the axon and the nucleus will have these glycoproteins in common. We addressed this issue by using galactosyltransferase and UDP-$^3$H-galactose to label and identify the glycoproteins in three regions of *Aplysia* neurons: axoplasm, extruded from nerves; nuclei, isolated by manual dissection of single neurons; and cytoplasm, obtained after removal of nuclei. At least 21 glycoproteins were labeled by this procedure; several, at 200, 180, 83, 76, and 66 kDa, from the nucleus and axoplasm comigrated after SDS-PAGE. Radiolabeled galactosyl-*N*-acetylglucosaminitol was released from the glycoproteins by base/borohydride, thereby verifying the presence of O-GlcNAc. Comparison of the 83 kDa glycoprotein from the nucleus and axoplasm revealed that both were soluble, had multiple O-GlcNAcs, and were bound to WGA. Thus, the 83 kDa constituent is a good candidate to use the axonal transport/nuclear import pathway.**

***[Key words: O-linked* N*-acetylglucosamine glycoproteins, nucleus, axoplasm, galactosyl transferase, neurons,* Aplysia*]***

In previous studies, single O-linked *N*-acetylglucosamine (O-GlcNAc) units were found on glycoproteins rapidly transported along axons in the CNS of *Aplysia californica* (Gabel et al., 1989) and also at growth cones of *Aplysia* neurons growing *in vitro* (Ambron et al., 1989). These were the first descriptions of O-GlcNAc–containing glycoproteins in the nervous system, but neither the number of glycoproteins nor their intracellular associations were determined. Studies on non-neuronal tissues have shown that this novel protein–saccharide linkage is widely conserved in eukaryotic cells and that it occurs on both membrane-bound and soluble glycoproteins (see Hart et al., 1989, for review). Among the latter are several RNA polymerase II transcription factors (Jackson and Tjian, 1988), several proteins associated with chromosomes (Kelly and Hart, 1989), a cofactor involved in protein translation (Datta et al., 1989), protein 4.1 (Inaba and Maede, 1989), and, most recently, the synapsins (Luthi et al., 1991). While no function has yet been ascribed to this modification, the O-GlcNAc can recycle. One intriguing possibility, therefore, is that O-glycosylation competes with phosphorylation for the serine/threonine sites on the polypeptide chain (Haltiwanger et al., 1992).

The presence of proteins that contain O-GlcNAc in the axon and nucleus, mentioned above, takes on added significance given that a pathway was recently discovered in neurons that conveys axoplasmic proteins through the axon to the cell body and then into the nucleus (Ambron et al., 1992). The pathway has been postulated to convey macromolecular signals from the axon periphery to the nucleus (Ambron et al., 1992) and it is attractive to think that these signals are O-GlcNAc–containing glycoproteins. An important step in testing this hypothesis would be to show that the same O-GlcNAc–glycoproteins are found in both the nucleus and axon.

We used *Aplysia* neurons in these experiments because their large size and well-defined, anatomically distinct regions make them amenable to manipulations that are difficult or impossible in mammalian neurons. Thus, nuclei and cytoplasm can each be isolated by manual dissection (Ambron, 1982) and axoplasm can be extruded from nerves (Sherbany et al., 1984). The enzyme galactosyltransferase and UDP-$^3$H-galactose (Torres and Hart, 1984) were used to determine the number of different O-GlcNAc–containing glyoproteins present within neurons. The enzyme transfers $^3$H-galactose to the O-GlcNAc moiety and is capable of detecting O-GlcNAc–containing glycoproteins that are present at very low levels. Glycoproteins with O-GlcNAc moieties that are inaccessible to the enzyme will not be labeled, however. After the reaction, we determined how each of the major radiolabeled glycoproteins was distributed among the various intracellular compartments. At least 21 O-GlcNAc polypeptides were found, five of which were present in both the nucleus and axoplasm.

## Materials and Methods

*Aplysia californica,* weighing 50–250 gm (supplied by Marinus, Long Beach, CA), were maintained in aerated, filtered Instant Ocean (Aquarium Systems, Eastlake, OH), with seaweed as food.

### *Isolation of nuclei and cytoplasm and extrusion of axoplasm*

The central nervous system was removed from the animal and pinned in a Sylgard-coated dish at 4°C containing sterile-filtered artificial seawater supplemented with amino acids and vitamins (culture medium, CM; Eisenstadt et al., 1973) and 0.01% phenylmethylsulfonyl fluoride.

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The sheath overlying each major ganglion was surgically removed and the large neuronal cell bodies were dissected from the neuropil (Ambron, 1982). Isolated cell bodies were teased open with fine needles and nuclei that emerged intact were manually separated from the cytoplasm and removed with a micropipette. After discarding the external envelope, cytoplasm was collected. All tissue samples were stored at −80°C. To obtain axoplasm, the right connective and nerves P7, P8, and P9 were severed from the ganglia and axoplasm was extruded into CM at 4°C by pulling the nerve or connective gently through the tines of forceps (Sherbany et al., 1984). In some experiments, axoplasm and isolated nuclei were separated into soluble and membrane fractions by centrifugation at 125,000 × *g* for 15 min at 4°C in a Beckman airfuge. The pellet was washed once with CM and the supernatant was combined with the original as the soluble fraction.

### *Morphological techniques*

Isolated nuclei were fixed with 2% glutaraldehyde, 3% paraformaldehyde in CM and were collected by centrifugation at 700 rpm for 8 min. Pelleted nuclei were washed three times with 0.1 M sodium cacodylate (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated in an ascending ethanol series, put into propylene oxide, and embedded in Epon (LX-112; Ladd, Burlington, VT). Silver–gold sections were stained for 5 min each in saturated uranyl acetate in 50% ethanol and then in Reynold's lead citrate. Sections were examined using a Phillips 100C electron microscope.

### *Galactosyltransferase labeling of subcellular fractions*

The reaction mixture contained approximately 20 μg protein from axoplasm, cytoplasm, or nuclei in 50 μl of CM, plus 2.5 mM $MnCl_2$, 2.5 mM ATP, and 2–5 U of either human milk (Boehringer–Manheim, Indianapolis, IN) or bovine (Sigma, St. Louis, MO) galactosyltransferase, in a total volume of 70 μl. In some experiments the enzyme was autogalactosylated prior to use (Torres and Hart, 1984). The reaction was initiated by adding 5–10 μCi of UDP-$^3$H-galactose (20 Ci/mmol; New England Nuclear, Boston, MA) to the reaction mixture. The samples were incubated for 2 hr at 21°C and the reaction was terminated by the addition, on ice, of 1 mM UDP-galactose and 10% trichloroacetic acid (TCA). The mixture was centrifuged and the pellet washed successively with 5% TCA, 50 mM Tris-HCl (pH 6.9), and ether. The final pellet was dried and extracted into sample buffer (Laemmli, 1970) by sonication and heating at 70°C for 15 min. After clarification, the supernatant was either subjected to SDS-PAGE or applied to a Sephadex G-50 column for analysis. SDS-PAGE indicated that the human and bovine enzymes recognized the same glycoproteins, so they were used interchangeably.

### *Polyacrylamide gel electrophoresis*

Samples were electrophoresed on a 10% polyacrylamide slab gel in SDS using the discontinuous method of Laemmli (1970). Prestained MW standards (Bio-Rad, Richmond, CA) were used to calibrate the gels and for comparison between gels; the mobility of each standard was corrected for the presence of the stain. Gels were dried and exposed to preflashed Kodak XAR film at −80°C. An enhancing screen (X-Omat, Kodak) was used for iodinated samples.

### *Iodination of proteins*

$Na^{125}I$ (500 μCi) was added to protein in 400 μl of 0.1 M phosphate buffer (pH 7.5) followed immediately by 30 μl of chloramine-T (2.5 mg/ml; Sigma). The mixture was vortexed for 60 sec and the reaction terminated with 30 μl of Na bisulfite (10 mg/ml) and 5 μl of KI (100 mg/ml). Iodinated protein was obtained in the excluded volume after gel filtration on a column of Sephadex G-25. The protein was recovered by acetone precipitation, and the individual radiolabeled polypeptides were resolved by SDS-PAGE.

### *Analytical techniques*

$^3$H-galactose–labeled glycoproteins in SDS sample buffer were chromatographed on a column of Sephadex G-50 eluted with 0.5% SDS in 50 mM Tris-HCl (pH 7.6). Excluded radiolabeled proteins were pooled, lyophilized, separated from SDS by acetone precipitation, and analyzed.

*β-Elimination* (Gabel et al., 1989). Precipitated glycoproteins were dissolved in 0.5 ml of 50 mM NaOH containing 1 M $NaBH_4$ and the mixture was incubated overnight at 45°C. After acidification, borate was removed by evaporation with methanol and the residue was dissolved in water and applied to a 1 ml column of Dowex 50 wx8 ($H^+$). The column was eluted with water and the eluate was dried under vacuum.

*Paper chromatography.* Reaction products were spotted onto Whatman #1 paper and the chromatographs developed in the descending direction with ethyl acetate/pyridine/acetic acid/water (5:5:1:3) (system 1) or ethyl acetate/pyridine/water (8:2:1) (system 2). Lanes containing radioactive material were cut into 1 cm segments and each segment was counted by liquid scintillation.

### *Preparation of the Gal-GlcNAcitol standard*

UDP-$^3$H-galactose (5 μCi; New England Nuclear) and 6 mU of galactosyltransferase were incubated with 1 mM GlcNAc under the conditions described above. When the reaction was complete, the mixture was applied to a 1 cm Amberlite column, which was eluted with 10 ml water. The eluate was concentrated and chromatographed with solvent system 2. The lane was cut into segments and each segment was eluted with water. Scintillation counting revealed two peaks of radioactivity. The most distal material ran just behind lactose and was pooled, concentrated, and treated with base/borohydride. The product was chromatographed with solvent system 1 where it was found to migrate with galactosyl-*N*-acetylglucosaminitol (Gal-GlcNAcitol) obtained by β-elimination of galactose-labeled glycoproteins from liver (Torres and Hart, 1984).

## Results

### *O-GlcNAc–containing glycoproteins of the nucleus, cytoplasm, and axoplasm*

Glycoproteins that contain GlcNAc in an O-linkage to serine/threonine can be conveniently labeled using galactosyltransferase and UDP-$^3$H-galactose (Holt and Hart, 1986). We used this approach to identify individual O-GlcNAc–containing glycoproteins in three regions of the neuron: axpolasm, obtained by gentle extrusion of axons that course within the large right pleuroabdominal connective; nuclei, isolated by manual dissection of neurons removed from ganglia of the CNS; and cytoplasm, obtained from dissected cells after the nucleus and external membrane had been removed (see Materials and Methods). Each cell fraction was incubated with galactosyltransferase, UDP-$^3$H-galactose, and $Mn^{2+}$ for 2 hr at 21°C. Time-course experiments established that incorporation of $^3$H-galactose into glycoprotein under these conditions reached a maximum at about 2 hr; with longer times the amount of product diminished. The reaction was terminated by adding excess unlabeled UDP-galactose and TCA was added to precipitate macromolecules. The precipitate was extracted with organic solvents to remove lipid. *Aplysia* glycolipids contain GlcNAc (Sherbany et al., 1984), but no radioactivity was found in the lipid fraction. The lipid-depleted proteins were extracted into SDS and resolved by SDS-PAGE and fluorography.

We did not observe incorporation when tissue was omitted from the incubation mixture (Fig. 1). A distinct and reproducible pattern was obtained from each cell fraction, however. As expected, since it is the biosynthetic center of the cell, the cytoplasm contained the greatest number of $^3$H-glycoproteins, and many of these were also present in the axoplasm and the nucleus. Even so, the patterns from the three fractions were different, reflecting the ability of the neuron to direct individual polypeptides to their appropriate cellular compartment. For example, three $^3$H-glycoproteins at approximately 110, 67, and 50 kDa (circles, Fig. 1) were greatly enriched in the axoplasm relative to the cytoplasm. Presumably these constituents have a role specific to the axon. We were interested to see a subset of glycoproteins, at about 200, 180, 83, 76, and 66 kDa, that were present in the nucleus and axoplasm (arrowheads, Fig. 1). One

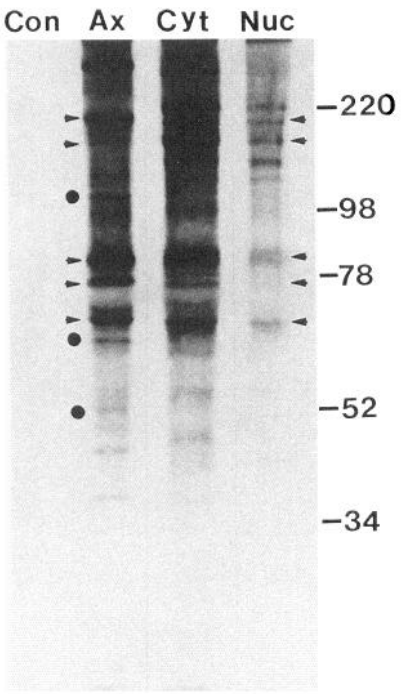


*Figure 1.* Identification of O-GlcNAc–containing glycoproteins among the regions of the neuron. Axoplasm (*Ax*), cytoplasm (*Cyt*), and nuclei (*Nuc*) were isolated and incubated in the presence of galactosyltransferase and UDP-$^3$H-galactose as described in Materials and Methods. The control (*Con*) contained enzyme, but no tissue. The reaction was terminated and the samples were heated in SDS and applied directly to a 10% polyacrylamide slab gel for SDS-PAGE and fluorography. The *circles* indicate glycoproteins that are greatly enriched in the axoplasm, and the *arrowheads*, those that are present in both axoplasm and nuclei.

of these, at 83 kDa, was consistently one of the more heavily labeled bands.

Nuclei from non-neuronal cells are known to contain both soluble and membrane-bound O-GlcNAc–containing glycoproteins (Hanover et al., 1987; Holt et al., 1987; Jackson and Tjian, 1988), but axoplasm has not been examined previously. The glycoproteins recognized by the galactosyltransferase comprise only a small subset of the total polypeptides since silver-stained gels of these fractions contained so many polypeptides that it was not even possible to identify any of the putative O-GlcNAc glycoproteins (not shown). This is similar to the situation in liver cells (Holt and Hart, 1986).

One concern in these experiments was that the glycoprotein pattern from the axoplasm of the right connective might not be representative of nerves in general because the connective is dominated by the very large axon of neuron R2. Extrusion and labeling of axoplasm from nerves throughout the animal, however, yielded the same pattern of $^3$H-glycoproteins and in subsequent experiments we routinely combined axoplasm from connectives and nerves.

### *Characterization of isolated axoplasm and nuclei*

Axoplasm extruded by our method is a translucent, syrupy material containing some membranous organelles, but lacking much of the cytoskeletal network. Biochemical studies indicated that extruded axoplasm is largely free of contamination by glial and connective cell constituents (Sherbany et al., 1984). Thus, the major $^3$H-glycoproteins from the axoplasm that migrated on SDS-PAGE with those from isolated nuclei were not contaminants from glial cells.

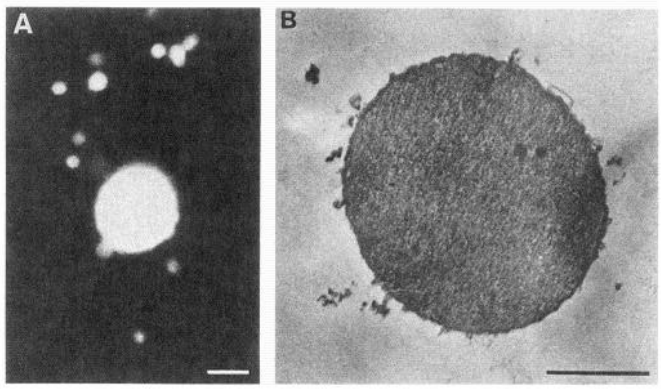


*Figure 2.* Examination of isolated nuclei. Nuclei from *Aplysia* neurons were isolated by manual dissection and cleaned of adherent cytoplasm as described in Materials and Methods. In *A*, a typical preparation of isolated nuclei was fixed, exposed to bisbenzamide, and examined by fluorescence microscopy. The nuclei are fluorescent, indicating that they contain DNA and are intact. *B*, Electron microscope micrograph of a section through a representative isolated nucleus. The nuclear membrane is intact and there are few adherent organelles. Scale bars: *A*, 40 μm; *B*, 50 μm.

The neuronal nuclei used in our studies were isolated individually by manual dissection and cytoplasm was removed by gentle washing with iso-osmolar culture medium. Since the cytoplasm of most *Aplysia* neurons contains bright orange pigment granules, the nuclei were considered free of cytoplasm when no more pigment was visible by light microscopy. Two kinds of evidence support this contention. First, analysis of data from previous studies on the distribution of newly synthesized, membrane-associated glycoconjugates in dissected neurons (Ambron et al., 1982; Sherbany et al., 1984) shows that only 4.0 ± 1.2% (mean ± SD; $n$ = 11) of the glycoconjugates were associated with isolated nuclei. Since we used the same isolation procedure, the nuclei in our experiments should be almost free from contamination by vesicles and other organelles. Second, examination of isolated nuclei by electron microscopy shows them to be largely free of attached cytoplasm (Fig. 2). We also took precautions to ensure that the nuclei were intact; broken nuclei are sticky due to exposed DNA and were discarded. In addition, the stain bisbenzamide was used in some experiments to confirm that nuclei retained their DNA (Fig. 2).

### *Nuclear and axoplasmic glycoproteins contain O-GlcNAc*

While the human and bovine galactosyltransferases incorporate $^3$H-galactose into glycoproteins that contain O-GlcNAc, these enzymes can also galactosylate GlcNAc units of N-linked oligosaccharides. Consequently, it is necessary to verify that the $^3$H-galactose–labeled glycoproteins from axoplasm and nuclei contained O-GlcNAc. This was accomplished by characterizing the radioactive products. First, the radiolabeled glycoproteins were acid precipitated, extracted into SDS, and subjected to gel filtration on Sephadex G-50 (Fig. 3*A,B*). As expected, both the nuclear and axoplasmic fractions yielded a peak of excluded radioactivity. Interestingly, a control consisting of axoplasm and UDP-$^3$H-galactose, but no exogenous enzyme, also gave an excluded peak (not shown), suggesting that there was an endogenous galactosyltransferase. This possibility is now being explored.

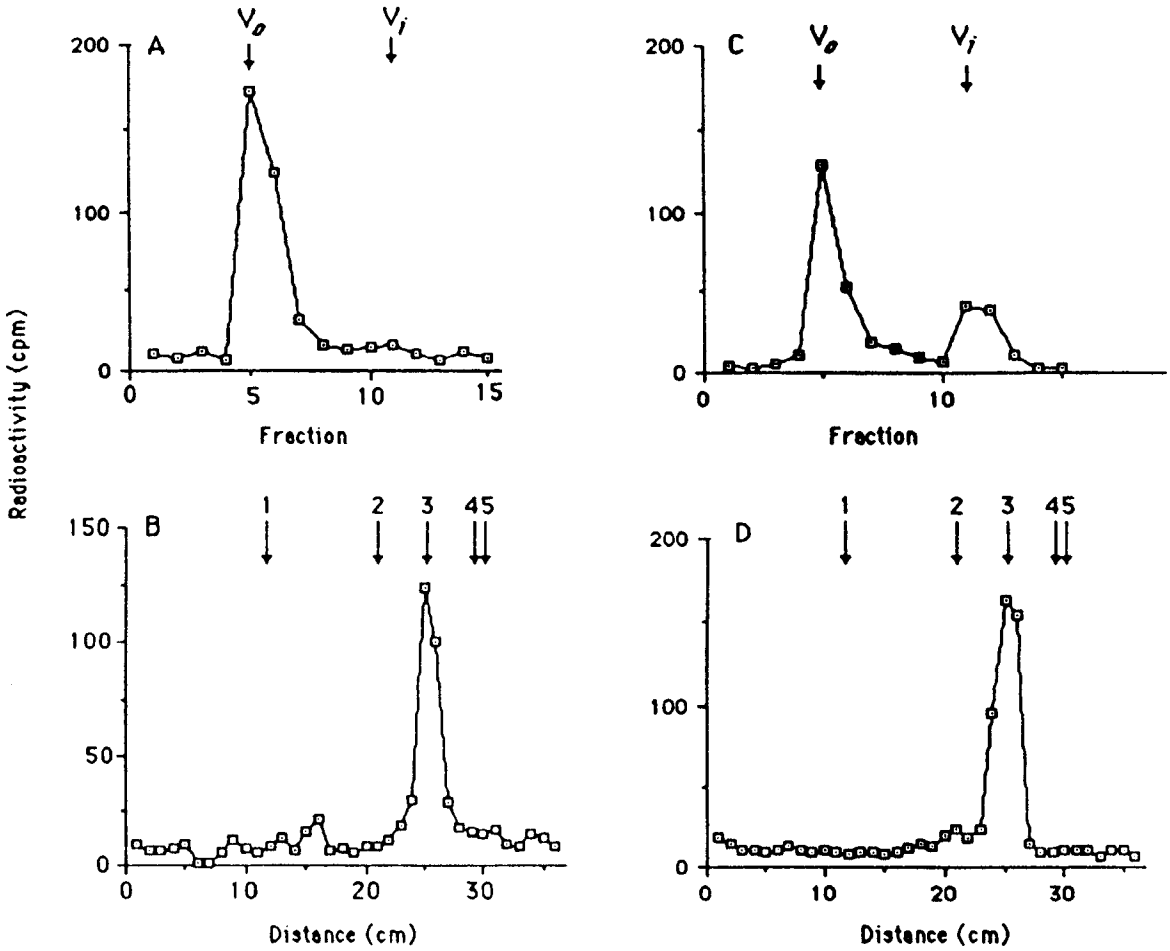


*Figure 3.* Characterization of the radioactive product formed after the galactosyltransferase-catalyzed incorporation of UDP-$^3$H-galactose into glycoprotein. After incubation with galactosyltransferase and UDP-$^3$H-galactose, the radiolabeled glycoprotein from isolated nuclei (*A*) and axoplasm (*C*) was applied to a column of Sephadex G-50. The $^3$H-glycoprotein in the excluded ($V_o$) fraction from both columns was pooled and treated with base/borohydride (see Materials and Methods), and the reaction products were resolved by paper chromatography in solvent system 1. A single radiolabeled product is obtained from both the nuclei (*B*) and axoplasm (*D*). The product has the same migration as authentic galactosyl-*N*-acetylglucosaminitol (*3*) and is completely separated from UDP-galactose (*1*), lactose (*2*), galactose (*4*), and galacitol (*5*).

The $^3$H-glycoproteins excluded from the column were recovered by acetone precipitation and treated with base/borohydride to release O-linked carbohydrates. The treated material then was placed over a cation exchange column: glycoproteins and glycopeptides will bind to the column while neutral or negatively charged O-linked carbohydrates pass through. We found that on average 84% ($n = 6$) of the radioactivity from axoplasm and 75% ($n = 2$) from nuclei passed through the column.

The next step was to determine whether the $^3$H-galactose was added to GlcNAc on the polypeptide; if so, then the base/borohydride-released material should contain $^3$H-Gal-GlcNAcitol. Paper chromatography showed this to be the case (Fig. 3*B,D*): most of the radiolabeled material from the eluate of both axoplasm and nucleus migrated with authentic Gal-GlcNAcitol in the two solvent systems, one of which is shown. The radiolabeled compound from the two sources was completely separated from UDP-$^3$H-galactose and $^3$H-galacitol. The latter product would result if the enzyme added galactose in an O-linkage directly to the polypeptide. The absence of radioactivity at or near the origin of the chromatogram indicated that larger O-linked oligosaccharides were not present. We conclude from these studies that most of the major radiolabeled polypeptides on the gel from the axoplasm and nucleus (Fig. 1) contain O-linked GlcNAc to which $^3$H-galactose was added by the galactosyltransferase.

### *Identification of a glycoprotein present in both the nucleus and axoplasm*

The comigration on SDS-PAGE of five O-GlcNAc glycoproteins from the nucleus and axoplasm (Fig. 1) was of interest since proteins with a dual localization are obvious candidates to utilize the pathway that conveys proteins through the axon to the nucleus (Ambron et al., 1992). One characteristic of the transported constituents is that they are soluble. We therefore separated nuclei and axoplasm into membrane and soluble fractions by centrifugation and labeled each with galactosyltransferase and UDP-$^3$H-galactose. SDS-PAGE revealed that the 83 kDa O-GlcNAc–containing glycoprotein was the most prominent constituent in the soluble fraction (not shown).

The 83 kDa glycoprotein is one of the major galactosyltransferase substrates (Fig. 1) and may contain multiple O-GlcNAc units. Such glycoproteins often bind to WGA (Jackson and Tjian, 1988) and this would afford us another way of comparing the axoplasmic and nuclear components. Soluble axoplasm from 20 connectives and nerves and the soluble fraction from approximately 60 nuclei were collected. The glycoproteins were

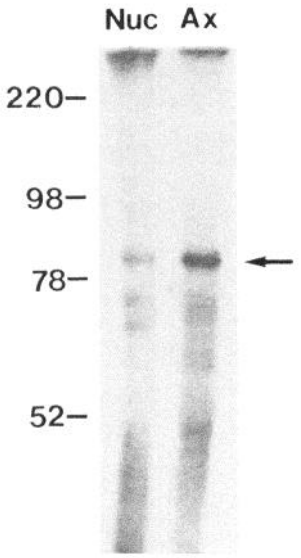


*Figure 4.* WGA affinity chromatography showing that the nucleus and axoplasm have an O-GlcNAc–containing glycoprotein in common. The soluble fraction from the nucleus (*Nuc*) and axoplasm (*Ax*) in CM were applied separately to a column of WGA-agarose. The column was washed with CM and the bound glycoprotein was eluted with 0.2 M GlcNAc and iodinated with $^{125}$I. The radiolabeled glycoproteins were separated by SDS-PAGE and the bands were detected by autoradiography. $^{3}$H-glycoproteins from axoplasm, labeled by incubation with galactosyltransferase and UDP-$^{3}$H-galactose, were run on the same gel. The $^{3}$H-glycoproteins were not visible after the 1 d exposure required for the iodinated glycoproteins, but the position of the 83 kDa constituent was determined after a longer exposure and is indicated by the *arrow*.

not labeled with galactosyltransferase since galactosylation of the GlcNAc blocks binding to WGA. Instead, the glycoproteins were applied directly to WGA-agarose. The column was washed and the bound glycoproteins eluted with 0.2 M GlcNAc. To facilitate identification of the bound glycoprotein, the material was iodinated and subjected to SDS-PAGE (Fig. 4). The nuclear and axoplasmic fraction each contained several iodinated glycoproteins. The predominant labeled band in both comigrated and also ran with the 83 kDa O-GlcNAc–containing glycoprotein that had been labeled by exogenous galactosyltransferase and run on the same gel (Fig. 4, arrow). Evidently, the 83 kDa species is the only major substrate for galactosyltransferase that is soluble and that contains a sufficient number of O-GlcNAc units to bind to WGA. There were other radiolabeled bands on the gel, but these were present in much smaller amounts and were in either the nucleus or axoplasm, but not in both.

## Discussion

### *Identification of glycoproteins containing O-GlcNAc in* Aplysia *neurons*

Bovine and human galactosyltransferases recognize a relatively small number of glycoproteins in the nucleus, cytoplasm, and axoplasm of *Aplysia* neurons. We characterized the galactose acceptor on the glycoproteins by base/borohydride treatment and column and paper chromatography. Between 75% and 80% of the $^{3}$H-galactose incorporated into glycoproteins from both the nucleus and axoplasm was recovered as Gal-GlcNAcitol, indicating that most of the glycoproteins contain GlcNAc attached to the polypeptide in an O-linkage. As expected, based on previously reported experiments, there was no incorporation into larger O-linked chains (Gabel et al., 1989). N-linked oligosaccharides containing GlcNAc are present on several *Aplysia* membrane glycoproteins and these may account for the remaining incorporated $^{3}$H-galactose; these were not analyzed.

Galactosyltransferases are very sensitive detectors of O-GlcNAc moieties (Jackson and Tjian, 1988), but the enzymes can also be autogalactosylated, thereby leading to potential misinterpretation of autoradiographs. We avoided this problem by doing control incubations (e.g., Fig. 1, lane 1) and by using enzyme that had been autogalactosylated with nonradioactive UDP-galactose. In addition, both the human and bovine enzymes, which have different molecular weights, yielded the same pattern of glycoproteins from *Aplysia* neurons. Manipulation of the incubation conditions, for example, by incubating at 37°C rather than 21°C, did not yield additional labeled species. Nevertheless, we may not have identified all of the O-GlcNAc–containing glycoproteins in these cells because some might be inaccessible to the enzyme or be capped by other sugars. The latter possibility is suggested by our finding that radiolabeled galactose was incorporated into macromolecules in axoplasm in the absence of exogenous galactosyltransferase. We have characterized the radiolabeled products and found that the galactose was indeed added to O-GlcNAc moieties on glycoproteins. The critical issue that needs to be addressed is whether or not the endogenous galactosyltransferase is in the axoplasm. There is precedence for axonal glycosyltransferases since *N*-acetylgalactosamine injected into the giant axon of *Aplysia* is incorporated into axonal glycoproteins (Ambron and Treistman, 1977).

### *The distribution of O-GlcNAc–containing glycoproteins in the various regions of the neuron*

The primary objective of the present work was to explore the possibility that nuclei and axoplasm have glycoproteins in common. This type of analysis can only be carried out using *Aplysia* neurons. Granted, nuclei can be isolated from many cell types, but pure axoplasm can be obtained only from giant invertebrate axons. Thus, only in *Aplysia* is a direct comparison between axoplasm and nuclei possible. Several O-GlcNAc glycoproteins were found in both the nucleus and axoplasm. One of these was an 83 kDa constituent that was a good substrate for galactosyltransferase, comigrated on SDS-PAGE, was soluble, and possessed a sufficient number of O-GlcNAC moieties to bind to WGA. Its presence in two distinct intracellular compartments of the neuron may be significant. One explanation for the dual localization would be that the glycoprotein is an enzyme or factor that is required in both locations. A more provocative idea is that this glycoprotein is transported through the axoplasm to the nucleus. Three lines of evidence support this idea. First, a retrograde transport/nuclear import pathway is present in *Aplysia* neurons (Ambron et al., 1992). The initial demonstration of this pathway was achieved using exogenous proteins, but there is now evidence that endogenous proteins use the pathway as well (R. Schmied, C. C. Huang, R. T. Ambron, and D. A. Ambron, unpublished observations).

Second, a nuclear import signal sequence is required for proteins larger than about 50 kDa to gain entry into the nucleus (Peters, 1986). Thus, the 83 kDa glycoprotein in the nucleus must have such a sequence. Since the nuclear and axoplasmic glycoproteins have the same molecular weight, it is reasonable to conclude that the 83 kDa axoplasmic constituent has this sequence also. The presence of such a sequence is very important because such signal sequences mediate access not only to the nuclear import apparatus, but also to the retrograde transport

system (Ambron et al., 1992; Schmied, Huang, Ambron, and Ambron, unpublished observations). We would predict, therefore, that the 83 kDa constituent uses the transport/import pathway.

Third, we have generated an affinity-purified antibody to the nuclear localization signal that mediates both transport and import. When tested against *Aplysia* nervous tissue, the antibody recognized an 83 kDa protein (Ambron et al., 1992). Whether or not this constituent is the 83 kDa O-GlcNAc–containing glycoprotein is presently under investigation. The identification of endogenous proteins that use the transport/import pathway will be useful in elucidating the role of this pathway in the neuron. We proposed that the transported constituents comprise a signaling system that links the axon periphery to the protein-synthesizing machinery in the cell body, and the presence of O-GlcNAc–containing transcriptional factors in axons would be a confirmation of this idea.

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