Tagging Secretory and Membrane Proteins with a Tyrosine Sulfation Site

TYROSINE SULFATION PRECEDES GALACTOSYLATION AND SIALYLATION IN COS-7 CELLS*

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Sulfation of proteins on tyrosines is a late Golgi modification that can be used to label proteins with [35S]sulfate for the analysis of post-Golgi transport. To extend the use of this modification to proteins not naturally sulfated, we fused a tyrosine sulfation site, the carboxy-terminal nonapeptide of cholecystokinin precursor, to the carboxyl terminus of two normally unsulfated proteins: aβ-proteinase inhibitor, a secretory protein, and subunit H1 of the asialoglycoprotein receptor, a type II membrane protein. The tagged proteins were efficiently sulfated in transfected COS-7 and Madin-Darby canine kidney cells. Specifically in COS-7 cells, the proteins were sulfated before they were galactosylated and sialylated and were converted to the mature forms with a half-time of approximately 2–3 min. This is in contrast to other cell types in which tyrosine sulfation was found to be virtually the last modification of the Golgi apparatus. Our results suggest that tyrosine sulfation occurs before the trans-Golgi in transfected COS-7 cells.

To study individual steps of intracellular membrane transport, two approaches have been widely used in mammalian systems: the use of conditions that result in the reversible accumulation of transported markers in a specific compartment from which transport can be followed upon shift to permissive conditions, and the use of compartment-specific modifications that allow labeling of proteins in transit and following their fate in pulse-chase experiments. For the characterization of post-Golgi transport, proteins can be reversibly accumulated in the trans-Golgi network by reducing the temperature to 20 °C (Matlin and Simons, 1983; Saraste and Kuismanen, 1984). However, depending on the cell type this transport block may not be effective for longer than 1–2 h. As a result, proteins which are slowly transported from the endoplasmic reticulum through the Golgi are not significantly accumulated in this period of time.

The sulfation of proteins on either carbohydrates or tyrosine residues is an established post-translational modification specific for the trans-Golgi (reviewed by Huttnet (1988)). Sulfation is particularly suitable for pulse labeling of proteins because [35S]sulfate is available at high specific radioactivity, is rapidly converted to the active precursor phosphoadenosine phosphosulfate and imported into the lumen of the Golgi, but is not incorporated into nascent proteins via methionine or cysteine (Huttner, 1984). Sulfation is considered to be one of the last Golgi modifications, occurring even after sialylation of attached oligosaccharides (Baeuerle and Huttner, 1987). Labeling of sulfated proteins with [35S]sulfate has been used successfully to study the sorting of proteins of the regulated and constitutive secretory pathways into distinct vesicle populations (e.g. Tooze and Huttner (1990) and Grimes and Kelly (1992)).

However, most proteins are not sulfated. Relatively few sulfated proteins have been identified so far, mainly secretory proteins and only few membrane proteins (Huttner, 1988). In this study we have extended the use of sulfation for transport studies to proteins not naturally sulfated by introducing a tyrosine sulfation site by in vitro mutagenesis of the encoding DNA. Tyrosine residues are sulfated by the enzyme tyrosylprotein sulfotransferase which recognizes tyrosines in exposed protein domains containing acidic amino acids (Horton et al., 1986; Rosenquist and Nicholas, 1993). We introduced a potential sulfation site into two proteins which are normally not labeled with [35S]sulfate: human aβ-proteinase inhibitor (A1Pi), a secretory protein, and subunit H1 of the human asialoglycoprotein (ASGP) receptor, a type II membrane protein. We have chosen the carboxy-terminal nonapeptide sequence of rat cholecystokinin precursor (proCCK) which is tyrosine sulfated in vivo (Adrian et al., 1986). When this peptide sequence was fused to the exposed carboxyl terminus of A1Pi or H1, the tagged proteins A1PiTS and H1TS were efficiently sulfated in transfected COS-7 and Madin-Darby canine kidney (MDCK) cells. Surprisingly, sulfation of these proteins in COS-7 cells could be observed before galactosylation and sialylation, indicating that the relative compartmentalization of the corresponding transferases in transfected COS-7 cells is unusual.

EXPERIMENTAL PROCEDURES

Materials—Endo-β-N-acetylgalcosaminidase H (Endo H), endo-β-N-acetylglucosaminidase F/N-glycosidase F (Endo F), neuraminidase (from Vibrio cholerae), and β-galactosidase (from bovine testes) were obtained from Boehringer Mannheim. Ricin agglutinin RCA120 insolubilized on cross-linked 4% beaded agarose (ricin-agarose) was from Sigma, and [35S]sulfate (carrier-free) was from Amersham. Cell culture reagents were purchased from Life Technologies, Inc. [3,5-3H]Tyrosine was from Amersham.

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‡ The abbreviations used are: A1Pi, aβ-proteinase inhibitor; ASGP, asialoglycoprotein; proCCK, cholecystokinin precursor; Endo H, endo-β-N-acetylgalcosaminidase H; Endo F, endo-β-N-acetylglucosaminidase F; MDCK, Madin-Darby canine kidney; PBS, phosphate-buffered saline.
DNA Constructs—Two complementary oligonucleotides were synthesized, CACGGAGAGACTAGATACCACCTTGAGAC and CAG- GATGGCTATGCTTCTTCTGCGCTGATC, which encode the 9 carboxy-terminal residues of rat cholecystokinin precursor followed by a stop codon and produce terminal protruding ends corresponding to a KpnI linker at the 5' and 3' ends, respectively. Both oligonucleotides were 5'-phosphorylated with polynucleotide kinase.

A KpnI site was introduced into the cDNA of the ASGP receptor subunit H1 (Spiess et al., 1985) following codon 279 by polymerase chain reaction using the antisense primer GTTGGTACCTGTCTCG-CAGAC (the KpnI site is underlined) in combination with a primer in the vector sequence. The amplified cDNA (from the HindIII to the KpnI site) was ligated into the vector plasmid pGEM4. Analogously, a KpnI site was introduced into the cDNA of A1Pi (variant M2; Brantly et al. (1988)) following codon 392 using the antisense primer CCGTGGTACCGGGTGGATCAC. The resulting plasmids were digested with KpnI and ScaI and ligated with the annealed synthetic oligonucleotides. The new 3' ends of the cDNAs were verified by DNA sequencing. To avoid mutations potentially introduced by Taq polymerase, most of the amplified sequences were replaced by corresponding segments from nonamplified plasmids by restriction enzyme digestion and ligation.

The cDNAs were subcloned into pECE (Ellis et al., 1985) for transient expression in COS-7 cells and into pSV2neo (with a Rous sarcoma virus promoter; provided by R. Gentz, Hoffmann-La Roche, Basel) for the production of stable MDCk cell lines. Stable lines expressing the H1 fusion protein were only obtained after nucleotides 1066-1278 of the original H1 cDNA (corresponding to most of its 3' untranslated sequence) had been added to the cDNA in the plasmid pSV2neo.

**Cell Culture and Transfection**—COS-7 cells and MDCk cells (strain II) were grown in minimal essential medium with 10% fetal calf serum, supplemented with 2 mm l-glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin sulfate. For transient expression, wild-type and mutant cDNAs subcloned in the expression plasmid pECE were transfected according to Cullen (1987) into COS-7 cells in 10-cm plates. To ensure uniform expression within each experiment, the cells were trypsinized 1 or 2 days after transfection, mixed, and seeded into 35-mm wells for use on the following day (i.e., 40-72 h after transfection). For stable transfection, MDCk cells were cotransfected with pSV2neo containing the cDNA of the ASGP receptor (which had been added to the cDNA in the plasmid pSV2neo) and polybrene and dimethyl sulfoxide according to Kawai and Nishizawa (1984). After 2 days, cells were split into selective medium containing 1 mg/ml G418-sulfate. After 14 days, resistant colonies were isolated and screened for expression by immunoblot analysis using a polyclonal antiserum directed against the ASGP receptor.

**Labeling with [3S]Methionine/Cysteine**—COS-7 cells expressing wild-type and mutant A1Pi were washed with phosphate-buffered saline (PBS), starved for 45 min in methionine-free medium (Selectamit kit, Life Technologies, Inc.), labeled for 30 min in 100 μCi/ml [35S]methionine and [35S]cysteine, washed twice with ice-cold PBS and lysed in lysis buffer (1% Triton X-100, 0.5% deoxycholate, 2 mm phenylmethylsulfonyl fluoride). The resulting lysates were preincubated with medium lacking methionine and cysteine for 45 min at 37 °C, and incubated with 2 milliunits of Endo H for 2 h at room temperature with 70 μl of a 50% (packed gel per volume) suspension of ricin-agarose. The agarose beads were pelleted for 5 min at 500 x g to separate free and ricin-bound protein. The supernatant was concentrated by precipitation in acetone (with 80 μg of bovine serum albumin as a carrier) and centrifuged briefly. The supernatant was taken off the protein A-Sepharose beads and incubated with shaking for 2 h at 37 °C. After centrifugation for 5 min at 10,000 x g at 4 °C the pellets were redissolved in SDS-sample buffer. The pelleted agarose beads were washed twice with binding buffer, followed by boiling in SDS-sample buffer to elute bound protein.

**Stoichiometry of Sulfation**—The stoichiometry of sulfation of A1PiTS was determined according to Huttner (1984). Transfected COS-7 cells were preincubated with medium lacking tyrosine for 30 min at 37 °C and labeled for 6 h in the same medium supplemented with 40 μCi/mI [3H]tyrosine. Labeled A1PiTS was immunoprecipitated from the medium and hydrolyzed in 0.2 m Ba(OH)2 at 110 °C for 20 h. The hydrolysate was neutralized with sulfuric acid and centrifuged. The supernatant was lyophilized, redissolved in 20 μl of electrophoresis buffer (7.8% acetic acid, 2.2% formic acid, pH 9), mixed with 100 μl of acetic, and centrifuged. The supernatant was dried, redissolved in electrophoresis buffer, and spotted onto a cellulose thin layer chromatography sheet (20 cm x 20 cm; 7 cm from the anionic edge). The sheet was wetted with the same buffer and run at 750 V for approximately 45 min to separate tyrosine and sulfotyrosine. [3H]Tyrosine and sulfo-[3H]tyrosine were quantified by liquid scintillation counting.

**RESULTS**

**Fusion of a Potential Tyrosine Sulfation Site to A1Pi and H1**—In Fig. 1 the sulfated nonapeptide sequence of proCCK is shown. It was chosen as a carboxyl-terminal tyrosine sulfation tag, because it is also carboxyl-terminal in proCCK, it contains two potentially sulfated tyrosines, and a synthetic peptide of this sequence has been shown to be a substrate for tyrosylprotein sulfotransferase (Niehrs et al., 1990a, 1990b). The secreted protein A1Pi has its carboxyl terminus exposed on the surface of the molecule as is evident from its crystal structure (Loebermann et al., 1984). Synthetic oligonucleotides corresponding to the nonapeptide sequence were fused to the cDNA...
of A1Pi via a linker segment encoding Gly-Thr which replaced the last two codons. It has previously been shown that the terminal 3 amino acids of A1Pi are not essential and can be deleted without affecting secretion (Brodbeck and Brown, 1992).

Subunit H1 of the human ASGP receptor is a single-spanning type II membrane protein. The carboxyl-terminal half of the protein forms a galactose binding domain homologous to a large family of calcium-dependent carbohydrate binding proteins (Drickamer, 1988; Geffen and Spiess, 1992). The homology does not extend beyond Cys-277 (which is involved in a disulfide bridge), suggesting that the carboxyl-terminal 14 residues are not essential for protein folding. The proCCK nonapeptide was fused to Thr-279 via the linker sequence Gly-Thr, thereby re-creating the tyrosine sulfation peptide were named H1TS and A1PiTS, respectively.

Expression and Sulfation of the Fusion Proteins in COS-7 Cells—In vivo sulfation of the fusion proteins was analyzed in transiently transfected COS-7 cells. A1PiTS, H1TS, and the wild-type proteins were expressed in COS-7 cells, labeled with either [35S]methionine or [35S]sulfate, immunoprecipitated, and analyzed by gel electrophoresis and fluorography (Fig. 2). After labeling for 30 min with [35S]methionine, A1PiTS was detected as a 50-kDa protein (Fig. 2A, lane 8) corresponding to the oligomannose-glycosylated precursor form of A1Pi (lane 1). Following a 2-h chase, both labeled proteins were recovered in the medium with a somewhat reduced mobility reflecting maturation to the complex glycosylated forms (lanes 2, 3, 9, and 10). After incubation for 2 h with [35S]sulfate, wild-type A1Pi was not labeled (lanes 5 and 7), whereas A1PiTS was efficiently labeled and could even be detected in a small aliquot of the total medium without immunoprecipitation (lanes 13 and 14). Radioactive A1PiTS could also be detected in the cells (lanes 11 and 12). The protein immunoprecipitated from the cells (lane 12) was predominantly of the mature form; however, a small fraction with a lower apparent molecular weight was also detectable (see below).

Wild-type H1 was expressed and labeled with [35S]methionine and [35S]cysteine, but did not incorporate [35S]sulfate (Fig. 2B, lanes 1–3). The fusion protein H1TS, however, was efficiently labeled with [35S]sulfate. While a considerable fraction of [35S]methionine/cysteine-labeled H1TS was present as the oligomannose-glycosylated precursor form of 40 kDa after a 3-h chase (lane 5), only the mature 46-kDa form was labeled with radioactive sulfate in this experiment (lane 6). Besides the proteoglycans (which accumulate on top of the gel), H1TS was the major sulfated protein synthesized in these transfected COS-7 cells, since it was easily detected in total cell lysates (lane 7).

To test whether the extension of A1Pi at its carboxyl terminus by the tyrosine sulfation peptide affected its transport behavior in the secretory pathway, we determined the kinetics of secretion of [35S]methionine-labeled A1Pi and A1PiTS. Transfected COS-7 cells were pulse-labeled for 30 min and chased for up to 2 h. Wild-type or mutant A1Pi was immunoprecipitated from the medium and the cell lysate, and quantitated by gel electrophoresis, fluorography, and densitometric scanning. As shown in Fig. 3, secretion of A1Pi and A1PiTS occurred with very similar kinetics: 50% secretion was observed after 40–45 min of chase. This suggests that the tyrosine sulfation tag did not significantly alter the transport rate of A1Pi from the endoplasmic reticulum to the cell surface.

To determine the stoichiometry of sulfation, COS-7 cells ex-
pressing AIPiTS were labeled for 6 h with [3H]tyrosine, secreted AIPiTS was immunoprecipitated, and hydrolyzed with Ba(OH)₂. Tyrosine and sulfotyrosine were separated by thin layer electrophoresis, and [3H]tyrosine and sulf-[3H]tyrosine were quantified by liquid scintillation counting. In four experiments, 11.0 ± 1.8% of labeled tyrosine residues were present in sulfated form (not shown). Since there is a total of 8 tyrosines in AIPiTS, this corresponds to approximately 1 sulfotyrosine/molecule. This value suggests that in average only one of the two potentially sulfatable tyrosines in the proCCK tag sequence is modified.

Two Intracellular Forms of AIPiTS Are Sulfated in COS-7 Cells—The introduction of a tyrosine sulfation tag allowed the determination of the transport kinetics of AIPiTS specifically from the site of sulfation to the cell surface. Based on previously published studies, we expected tyrosine sulfation to occur in the trans-Golgi network. Transfected COS-7 cells were pulse-labeled with [35S]sulfate for 7 min at 37°C and chased for up to 35 min. Cell-associated and secreted labeled AIPiTS was immunoprecipitated and analyzed by gel electrophoresis and fluorography, as shown in Fig. 4A. The results show that a precursor form of lower apparent molecular weight was sulfated. During the chase, this material was converted to the larger, mature form of the protein, which was finally released into the medium. Conversion of the precursor to the mature form occurred with a half-time of approximately 2–3 min.

Quantification of similar experiments (by densitometric scanning of both cellular forms versus secreted AIPiTS) yielded the kinetics of transport from the intracellular site of sulfation to the cell surface, as shown in Fig. 5. After a lag time of approximately 10 min the protein began to appear in the medium. After approximately 17 min of chase, 50% of the labeled AIPiTS was secreted.

When cells were incubated with [35S]sulfate for very short periods at 37°C, the lower form of AIPiTS was labeled first and was detected after 3 min of labeling (Fig. 4B). The higher, mature form appeared approximately 2 min later. Exocytic transport can be blocked specifically at the level of the trans-Golgi network by lowering the temperature to 20°C (Matlin and Simons, 1983; Saraste and Kuismanen, 1984). When COS-7 cells expressing AIPiTS were incubated at 20°C with [35S]sulfate (Fig. 4C), only the smaller labeled form was detected after 10 min of labeling (lane 1). After longer labeling periods, the higher molecular weight form appeared and increased in intensity with time (lanes 3, 5, and 7). This confirms that the lower form corresponds to a transient precursor of the higher form. Incubation at 20°C prevented secretion of AIPiTS during the first 60 min; only after 100 min was some mature protein released into the medium (lane 8). Sulfation and the modification(s) responsible for the change in electrophoretic mobility thus occur in a compartment before the inhibited

![Fig. 3. Secretion of [35S]methionine-labeled AIPi and AIPiTS from transfected COS-7 cells. Transiently transfected COS-7 cells were pulse-labeled for 30 min with [35S]methionine and chased for up to 120 min. AIPi (M) or AIPiTS (C) was immunoprecipitated from cell lysates and culture medium and analyzed by gel electrophoresis and fluorography. Secreted protein is expressed as percent of the total protein (intracellular plus secreted) as determined by densitometric scanning of the fluorograms. The mean of duplicate samples is shown.](image)

![Fig. 4. [35S]Sulfate labeling of different intracellular forms of AIPiTS in transfected COS-7 cells. Panel A, COS-7 cells expressing AIPiTS were pulse-labeled with [35S]sulfate for 7 min and chased for up to 35 min at 37°C. AIPiTS was immunoprecipitated from the cell lysates (C) and from the medium (M). Panel B, COS-7 cells expressing AIPiTS were labeled with [35S]sulfate for 1–7 min at 37°C, and AIPiTS was immunoprecipitated from the cell lysates (C). Panel C, cells were labeled with [35S]sulfate for up to 100 min at 20°C, and AIPiTS was immunoprecipitated from the cell lysates (C) and the medium (M). Samples were analyzed by gel electrophoresis and fluorography. For better visualization of the initially labeled material, lanes 1 and 2 of panel C were exposed longer than the other lanes.](image)
transport step, i.e. before exit from the trans-Golgi network.

**Sulfation of AIPiTS in COS-7 Cells Occurs before Galactosylation and Sialylation**—Human AIPi contains three asparagine-linked, biantennary oligosaccharide side chains (Mega et al., 1980). Different carbohydrate modifications were thus likely to account for the two sulfated forms of AIPiTS. To analyze the structural difference between the precursor and the final form, this indicates that sulfation occurred beyond the cis-Golgi. Deglycosylation with Endo F converted both forms to a single species of an apparent molecular mass of 44 kDa (lane 3), demonstrating that the two forms differ within their carbohydrate moieties. Similarly, when COS-7 cells expressing AIPiTS were incubated with tunicamycin (3 μg/ml) to inhibit N-glycosylation and then labeled with [35S]sulfate for 25 min at 20 °C, only a single radioactive species of 44 kDa was immunoprecipitated corresponding to the unglycosylated protein (not shown).

Of the two forms of [35S]sulfated AIPiTS only the larger one was sensitive to neuraminidase (Fig. 6, lane 5), indicating that the final form, but not the precursor, was sialylated. However, desialylation did not convert the larger form to the smaller one, but resulted in a protein of intermediate electrophoretic mobility. The two species migrated with indistinguishable mobility only when the sample was treated with neuraminidase in combination with β-galactosidase (lane 6). This indicates that the precursor form was neither sialylated nor galactosylated. This was confirmed using the galactose-specific lectin ricin. Both the precursor as well as the final sulfated form of AIPiTS were recovered in the supernatant after incubation with ricin-agaro-rose and centrifugation (lanes 7 and 8). After neuraminidase treatment, the desialylated final species was efficiently bound to ricin-agaro-rose (lanes 9 and 10) and served as a positive control for the assay.

**Sulfation of a Precursor of H1TS Occurs in COS-7 Cells, but Not in MDCK Cells**—To test whether tyrosine sulfation of ungalactosylated and unsialylated AIPiTS is due to the particular substrate glycoprotein analyzed, or is a general property of COS-7 cells, we analyzed the sulfation of H1TS in more detail in

**Fig. 6. Carbohydrate analysis of the sulfated forms of AIPiTS.** AIPiTS expressed in COS-7 cells was labeled with [35S]sulfate for 25 min at 20 °C and immunoprecipitated from the cell lysate. The immunoprecipitates were incubated with Endo H, Endo F, neuraminidase (Neu), neuraminidase and β-galactosidase (Neu + Gal), or no enzyme (−) as indicated (lanes 1−6). In addition, untreated (−) and neuraminidase-digested samples (Neu) were incubated with ricin-agaro-rose and bound free protein separated by centrifugation into a pellet (P) and supernatant fraction (S), respectively (lanes 7−10). Samples were analyzed by gel electrophoresis and fluorography.
Tagging Proteins with Tyrosine Sulfation Sites

In our study, we observed that incompletely processed precursor glycoproteins are already sulfated in COS-7 cells. Both tagged proteins A1P\textsuperscript{TS} and H1\textsuperscript{TS} behaved identically. The sulfated precursor of A1P\textsuperscript{TS} differs from the mature form exclusively in the structure of the oligosaccharides. The precursor is in a D-histidine and insensitive to neuraminidase and beta-galactosidase treatment, it is not recognized by the galactose-specific lectin ricin, and has the same electrophoretic mobility as the desialylated and degalactosylated mature form of the protein. As a result, the oligosaccharide structures of the precursor must be either (GlcNAc)\textsubscript{2}(Man)\textsubscript{3}GlcNAc or (GlcNAc)\textsubscript{2}(Man)\textsubscript{3}(GlcNAc)\textsubscript{3} (assuming biantennary structures as determined for A1P from serum). These carbohydrate structures are the products of mannosidase II and N-acetylglucosaminyltransferase II, respectively, which are considered to be medial-Golgi enzymes (Dunphy et al., 1981; Dunphy and Rothman, 1983; Goldberg and Kornfeld, 1983).

Our results indicate that the relative compartimentalization of the three modification enzymes galactosyltransferase, sialyltransferase, and tyrosylprotein sulfotransferase is different in COS-7 cells than in other cell types. No intermediate forms between the precursor and the final sialylated form could be observed. Conversion of the precursor to the final form occurred in the time span of a few minutes. These two observations point to a transport step between a Golgi compartment where sulfation takes place and a subsequent compartment where both galactosyltransferase and sialyltransferase are predominantly active. Tyrosine sulfation thus appears to occur before the trans-Golgi. Consistent with this conclusion, secretion of \(^{35}\text{S}\) sulfated A1P\textsuperscript{TS} from the cells was preceded by a lag period of approximately 10 min, which had not been observed in other cell types where only mature proteins are sulfated (Baeuerle and Huttner, 1987; Friederich et al., 1988; Régnier-Vigouroux et al., 1991).

The same fusion proteins which are sulfated as precursors in COS-7 cells are exclusively sulfated as the mature species in transfected MDCK cells. This shows clearly that the phenomenon observed in COS-7 cells is characteristic of that cell type rather than a property of the proteins analyzed. Different processing of secretory glycoproteins by different cells and tissues is considered to be due to qualitative and/or quantitative differences in the modification activities in the secretory pathway. Our results suggest that also the distribution of enzyme activities in the Golgi subcompartments can be different in different cell types.

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REFERENCES

Adrian, T. E., Domin, J., Baeerese Hamilton, A. J., and Bloom, S. R. (1986) FEBS Lett. 196, 5–8
Aratani, Y., and Kitagawa, Y. (1988) FEBS Lett. 235, 129–132
Baeuerle, P. A., and Huttner, W. B. (1987) J. Cell Biol. 105, 2655–2664
Breachy, M., Nikiwa, T., and Crystal, R. G. (1988) Ann. Med. Suppl. 6A, 84, 13–31
Brodbeck, R. M., and Brown, J. L. (1986) J. Biol. Chem. 261, 294–297
Cullen, B. R. (1987) Methods Enzymol. 152, 684–704
Dickman, K. (1988) J. Biol. Chem. 263, 9557–9560
Dunphy, W. G., and Rothman, J. E. (1983) J. Cell Biol. 97, 270–275
Dunphy, W. G., Fries, E., Urbani, L. J., and Rothman, J. E. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7453–7457
Ellin, L., Claeuser, E., Morgan, D. O., Edery, M., Roth, R. A., and Rutter, W. J. (1986) Cell 45, 721–732
Friederich, E., Fritz, H.-J., and Huttner, W. B. (1988) J. Cell Biol. 107, 1655–1667
Geffen, I., and Spiess, M. (1992) Int. Rev. Cyt. 137B, 181–219
Goldberg, D. E., and Kornfeld, S. (1985) J. Biol. Chem. 260, 3159–3165
Grimes, M., and Kelly, R. B. (1992) J. Cell Biol. 117, 539–549
Hertig, M., Foa, R., Gordon, J. I., and Strauss, A. W. (1986) Biochem. Biophys. Res. Commun. 141, 326–333
Hutner, W. B. (1984) Methods Enzymol. 107, 200–223
Huttner, W. B. (1986) Annu. Rev. Physiol. 50, 363–376
Kawar, S., and Nishizawa, M. (1984) Mol. Cell. Biol. 4, 1172–1174
Looeeman, H., Tokuoka, R., Deisenhofer, J., and Huber, R. (1984) J. Mol. Biol. 177, 531–556
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Matlin, K. S., and Simons, K. (1983) Cell 34, 233–243
Mega, T., Lujan, E., and Yoshida, A. (1980) J. Biol. Chem. 255, 4057–4061
Niehrs, C., and Huttner, W. B. (1990) EMBO J. 9, 35–42
Niehrs, C., Huttner, W. B., Carvalho, D., and Degryse, E. (1990a) J. Biol. Chem. 265, 9314–9318
Niehrs, C., Kraft, M., Lee, R. W. H., and Huttner, W. B. (1990b) J. Biol. Chem. 265, 8525–8532
Niehrs, C., Huttner, W. B., and Ruther, U. (1992) J. Biol. Chem. 267, 15938–15942
Rénvier-Vigouroux, A., Tsoze, S. A., and Huttner, W. B. (1991) EMBO J. 10, 3589–3590
Rose, P., Mantovani, S., Rosboch, R., and Huttner, W. B. (1992) J. Biol. Chem. 267, 12227–12232
Rosenquist, G. L., and Nicholas, H. B. (1993) Protein Sci. 2, 215–222
Roth, J., and Berger, E. G. (1982) J. Cell Biol. 93, 223–229
Roth, J., Taatjes, D. J., Lucocq, J. M., Weinstein, J., and Paulsen, J. C. (1985) Cell 43, 287–295
Saraste, J., and Kuismanen, E. (1984) Cell 38, 535–549
Slot, J. W., and Geuze, H. J. (1983) J. Histochem. Cytochem. 31, 1049–1056
Spires, M., Schwartz, A. L., and Lodish, H. F. (1985) J. Biol. Chem. 260, 1979–1982
Strous, G. J., Van Kerkhof, P., Willemsen, R., Geuze, H. J., and Berger, E. G. (1983) J. Cell Biol. 97, 723–727
Tsoze, S. A., and Huttner, W. B. (1990) Cell 60, 837–847