Immunocytochemical Localization of Galactosyltransferase in HeLa Cells: Codistribution with Thiamine Pyrophosphatase in trans-Golgi Cisternae

JÜRGEN ROTH and ERIC G. BERGER
Institute of Histology and Embryology, University of Geneva Medical School, Geneva, and Institute of Medical Chemistry, University of Berne, Berne, Switzerland

ABSTRACT An affinity-purified, monospecific rabbit antibody against soluble human milk galactosyltransferase was used to localize the enzyme in HeLa cells by immunofluorescence and by the protein A-gold technique at the electron microscope level. Specific immunofluorescence was observed in a juxtanuclear cytoplasmic region which was identified, on immunostained thin sections of low-temperature Lowicryl K4M-embedded HeLa cells, as Golgi apparatus. Label by gold particles was limited to two to three trans cisternae of the Golgi apparatus, indicating a compartmentalization of galactosyltransferase in the cisternal stack. Combination of preembedding thiamine pyrophosphatase cytochemistry with postembedding immunostaining for galactosyltransferase proved codistribution of the two enzymes. However, the acid phosphatase-positive, trans-most cisterna was negative for galactosyltransferase. The close topological association of both galactosyltransferase and thiamine pyrophosphatase (or nucleoside diphosphatase) suggests a concerted action of both enzymes in glycosylation.

Biosynthesis of complex type heteroglycans proceeds in two distinct stages in different cellular organelles: first, N-glycosylation involving the en bloc transfer of a lipid-linked oligosaccharide takes place in the rough endoplasmic reticulum (for recent review, see reference 27) where part of it is processed by glucosidases (6); thereafter, the glycoproteins move to the Golgi apparatus where N-linked glycans are further processed (10) before being elongated by sequential action of terminal glycosyltransferases which include N-acetyl-D-glucosaminyl-, galactosyl-, fucosyl-, and sialyltransferases (for review, see reference 26). On the basis of cell fractionation studies (3, 5, 11, 15) and autoradiographic (1, 19) evidence, chain elongation is assumed to occur in the Golgi apparatus. Accordingly, galactosyltransferase activity has frequently been selected as marker for Golgi fractions (see 3, 5, 8, 11, 14, 15, 26 for selected references). In order to obtain conclusive in situ evidence of the Golgi-association of galactosyltransferase (UDP-galactose:β-D-N-acetylglucosaminyl-protein β (1-4) transferase, EC 2.4.1.22) and to get insight into the glycosylation mechanism, an immunocytochemical study in HeLa cells was conducted. For this purpose the protein A-gold (pAg) technique as developed by Roth et al. (20, 22) was chosen. This postembedding staining method does not have the drawback of limited antibody penetration into cells characteristic of the preembedding labeling techniques. In addition, the use of gold particles as marker results in excellent and clear localization of the antigen to cellular organelles (23, 24). We report here the first ultrastructural localization of a glycosyltransferase, namely galactosyltransferase, and demonstrate its presence in a distinct Golgi subcompartment which is composed of two to three thiamine pyrophosphatase-positive trans-Golgi cisternae.

MATERIALS AND METHODS

Galactosyltransferase was purified from pooled human milk (9). The immunization schedule included six subcutaneous injections of 0.2 mg of galactosyltransferase emulsified in complete Freund's adjuvant into rabbits. Antibody production was monitored by an enzyme-linked immunosorbent assay (ELISA) (2). Monospecific antibodies were obtained by affinity purification on immobilized galactosyltransferase. Their specificity towards purified galactosyltransferase was checked by ELISA (2) and the immunoreplica technique essentially according to Towbin et al. (29), except that the antigen on the nitrocellulose sheet was developed using a protein A-peroxidase conjugate as described in reference 2.

HeLa cells (Microbiological Associates, Bethesda, MD) were grown in culture dishes in medium F11 (Gibco Laboratories, Grand Island, NY) supplemented with 10% calf serum until confluence of the monolayer was reached. Confluent monolayers were fixed with chilled acetone for 5 min, rinsed five times for 5 min each with PBS (0.15 M NaCl, 0.05 M phosphate buffer pH 7.3), and stored in PBS overnight at 4°C. The cells were incubated in situ with the galactosyltransferase antibody (100 μg/ml) for 2 h at room temperature, rinsed three times with PBS for 5 min each, and exposed to fluorescein isothiocyanate (FITC)-labeled protein A (0.1 mg/ml, Pharmacia Fine Chemicals, Uppsala, Sweden).
Swedish) for 1 h at room temperature. The cells were counterstained with 0.01% Evans blue and observed in a Leitz Ortholux fluorescence microscope equipped with a Ploemopak L2 illuminator. Specificity controls included (a) incubation with the galactosyltransferase antibody preabsorbed with an excess of purified galactosyltransferase followed by FITC-labeled protein A and (b) omission of the antibody incubation step.

For antigen localization on thin sections of resin-embedded cells by the pAg technique (20, 22), confluent monolayer cultures were fixed in 0.5% glutaraldehyde or in 2% formaldehyde with 0.1% glutaraldehyde in PBS for 30 min at room temperature, rinsed several times with PBS, and treated with 0.5 M NH4Cl in PBS for 45 min in order to block free aldehyde groups. After further rinses in PBS, the cells were incubated using a rubber policeman, pelleted by centrifugation, and encased in 1% agar. Since we were unable to localize the antigenic sites on thin sections of Epon-embedded cells, we performed embedding according to the recently developed low-temperature embedding procedure using the polar methacrylate mixture Lowicryl K4M (4, 12). This embedding protocol has been shown to provide better conditions for the preservation of antigenicity and cellular fine structure than Epon embedding (21).

Thin sections (65-80 nm) were mounted on nickel grids having a Parlodion-carbon film and processed for the pAg technique. Nickel grids with the attached thin sections were floated on a drop of PBS for 5 min, transferred to a drop of galactosyltransferase antibody (100 μg/ml) for 2 h at room temperature in a moist chamber, carefully washed with PBS, and further incubated with the pAg complex for 1 h at room temperature. After rinses with PBS and distilled water, sections were counterstained with 2% uranyl acetate (7 min) and lead citrate (45 s) and examined in a Philips EM 300 electron microscope at 60 kV.

In addition, monolayer cultures were labeled before embedding in order to visualize cell-surface galactosyltransferase. Unfixed cells or prefixed cells (fixed in 2% newly prepared formaldehyde in PBS for 5 min) were incubated with the galactosyltransferase antibody (100 μg/ml) for 1 h at 4°C, followed by pAg for 1 h at 4°C. After rinses with cold PBS, fixation with 2% glutaraldehyde in PBS for 30 min at 4°C and 1% OsO4, Cells were embedded in Epon.

Cytochemical controls included: (a) replacement of the antibody by an antibody preabsorbed with purified galactosyltransferase followed by the pAg complex, (b) omission of the antibody incubation step, and (c) incubation with the antibody followed by 1-h incubation with nonlabeled pA (0.1 mg/ml) and then by pAg complex.

The intensity of labeling expressed as number of gold particles/μm² of labeled trans-Golgi cisternae, remaining Golgi apparatus, lysosomes, mitochondria, cytoplasm (without lysosomes and mitochondria), and nucleus was evaluated on a total of 45 HeLa cells from three different confluent monolayers. Micrographs were taken on 70-mm film at the primary magnification of 25,000 (for the Golgi apparatus) or of 11,000 (for the other cellular compartments) calibrated with a carbon grating replica (2,160 lines/mm). The surface of the different compartments as well as the number of gold particles present over them was recorded on a graphic tablet (Tektronix, type 4973) connected to a microprocessor system (IMSAI, type 8080) programmed to calculate the number of particles/μm² of the compartment. (D. Bertrand and M. Amherdt, manuscript in preparation). Statistical comparison of the values was done with the Student’s t test.

Confluent cell cultures were fixed in 2% formaldehyde with 0.1% glutaraldehyde in PBS for 30 min, washed several times with 7% sucrose in PBS, and then rinsed in 7% sucrose overnight at 4°C. Cytochemical reactions were performed on monolayer cultures that had been frozen and thawed. Thiamine pyrophosphatase (TPPase) activity was demonstrated by the method of Novikoff and Goldfischer (17) (incubation time, 40 min at 37°C). Substrate omission and the addition of 10 mM sodium fluoride served as the cytochemical controls.

Acid phosphatase was localized as described by Novikoff (16) with cytofine-p5-monophosphate as substrate (incubation time, 15-40 min at 37°C). Controls consisted of incubation in a substrate-free medium or in medium containing 10 mM sodium fluoride. Afterwards, the cultures were washed with 7% sucrose, fixed with OsO4, and embedded in Epon.

Cells were incubated in situ for TPPase as described above. After washes with sucrose and without OsO4, fixation, low-temperature Lowicryl K4M embedding followed. Finally, thin sections were prepared and processed for galactosyltransferase localization as already described.

RESULTS

Galactosyltransferase purified from human body fluids by sequential affinity chromatography on N-acetylglucosamine-agarose and α-lactalbumin agarose yields a family of charge heteromers with similar molecular weights, all of them exhibiting comparable enzyme activity and substrate specificity (9). The structural basis of charge heterogeneity as revealed by analytical isoelectric focusing is unknown; the pattern may be characteristic and highly reproducible (compare Fig. 1 A with pattern published in reference 9). The affinity-purified rabbit antibody against human milk galactosyltransferase was tested for its specificity by several methods including enzyme inhibition, double immunodiffusion (our unpublished observations), and ELISA (2). The immunoreplica technique (29) was applied to purified and crude galactosyltransferase (Fig. 1 B and C) resolved on isoelectric focusing: replication of the galactosyltransferase pattern using crude human milk confirmed the specificity of the antibody used and showed that it is directed against all charge heteromers of galactosyltransferase.

Fig. 2 shows the immunofluorescence pattern of HeLa cells

![FIGURE 1 Immunoreplica of human milk galactosyltransferase resolved on isoelectric focusing. Purified enzyme was subjected to analytical isoelectric focusing in a pH gradient 3.5-9 as previously described (9). Lane A shows a Coomassie Blue stain of purified enzyme which is composed of at least nine charge heteromers. Lane B shows its corresponding immunoreplica (from a different gel). Lane C shows a replica from crude human milk resolved on isoelectric focusing.](image-url)
stained in situ. In each cell a fluorescent reticular or ribbonlike structure was seen in the juxtanuclear region. The immunofluorescence was specific for galactosyltransferase since it could be completely abolished under the different conditions of the cytochemical controls (Fig. 3).

The fine structure of HeLa cells after mild aldehyde fixation and low-temperature embedding in Lowicryl K4M was generally well-preserved. As shown in Fig. 4, a specific labeling for galactosyltransferase as revealed by the presence of gold particles was present only over the Golgi apparatus. Small vesicles present in this region were practically devoid of label. Sometimes, a low number of gold particles was scattered over the nucleus and the cytoplasm. However, as judged from the cytochemical controls, these gold particles and especially small-particle aggregates were due to a low degree of nonspecific background staining. In controls, as defined in Materials and Methods, the labeling by gold particles over the Golgi apparatus was abolished (Table I).

In cases where the Golgi apparatus was cut along the cis axis, the gold particle label was found only over two to three Golgi cisternae at the trans (inner maturing) side of the Golgi apparatus (Figs. 5-7). The cisternae in intermediate regions and at the cis side (Figs. 5 and 6) as well as the trans-most cisternae (Fig. 7) were not labeled. This labeling pattern was consistently found in all Golgi apparatus examined. No definite decision could be made as to whether the gold particle labeling was related to membranes or to the cisternal space of Golgi cisternae, since the best lateral resolution of the pAg technique as performed in this study is ~18 nm. Cell-surface galactosyltransferase immunoreactive sites were not detectable by light or electron microscopy.

In HeLa cells, reaction product for thiamine pyrophosphate activity was found only in two to three Golgi cisternae at the trans (inner maturing) side (Fig. 8). Acid phosphatase activity was found only in the trans-most cisterna adjacent to the Golgi stack and in lysosomes (Fig. 9). In Figs. 10-12, the double staining for TPPase and galactosyltransferase is illustrated. The gold particle label revealing galactosyltransferase immunoreactive sites was associated only with TPPase-positive trans-Golgi cisternae. Both enzymes were absent in the trans-most cisterna (Figs. 10 and 11).

**DISCUSSION**

Functional and structural heterogeneity of membranes of the Golgi apparatus has been demonstrated by biochemical studies on Golgi subfractions, measurement of lipid and enzyme content in isolated Golgi cisternae, by different histochemical techniques such as osmium impregnation, enzyme or carbohydrate histochemistry, and by thin-section and freeze-fracture electron microscopy (for comprehensive literature, see references 7, 16, 18, 28). This diversity suggests the existence of functional subcompartments within this complex organelle (25, 28). In the present work, we provide in situ evidence by immunoelectron microscopy that, in HeLa cells, galactosyltransferase immunoreactive sites are localized in two to three cisternae of the trans side of the Golgi apparatus. It should be borne in mind that all antigenic sites may not be preserved after aldehyde fixation and resin embedding for subsequent immunocytochemical localization with the pAg technique or might be present in amounts too low to be immunocytochemically demonstrable. With respect to possible antigen denaturation due to organic solvents and resins, the low-temperature embedding procedure employed in this study provides favorable conditions for antigen preservation and is superior to Epon embedding which was unsuccessfully applied for immunocytochemical localization of galactosyltransferase. Since the Golgi labeling pattern for galactosyltransferase was consistent and reproducible, we believe we are justified to assume that the observed concentration of gold particles on trans cisternae represents meaningful topographic specialization and an example of subcompartmentalization within the stack of Golgi cisternae.

**Table I**

| Antigalactosyltransferase* | 96.28 ± 4.75 | 0.87 ± 0.48 | 1.13 ± 0.52 | 2.01 ± 0.41 | 1.02 ± 0.59 | 1.89 ± 0.29 |
|----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Antigen-adsorbed antibody  | 0.46 ± 0.29 | 0.49 ± 0.27 | 0.29 ± 0.18 | 0.28 ± 0.09 | 0.10 ± 0.07 | 0.93 ± 0.22 |
| pAg alone                  | 0.18 ± 0.05 | 0.16 ± 0.07 | 0.15 ± 0.07 | 0.31 ± 1.1 | 0.08 ± 0.06 | 0.07 ± 0.03 |
| Antigalactosyltransferase-pA- | 0.21 ± 0.09 | 0.17 ± 0.05 | 0.14 ± 0.03 | 0.30 ± 0.9 | 0.10 ± 0.06 | 0.1 ± 0.04 |

* Data based on evaluation of a total of 45 cells from three different cultures. Student's t test analysis of data indicates significant differences (P < 0.001) in labeling intensity over trans-Golgi cisternae compared to other cellular compartments and control values.
several endoplasmic reticulum "marker" enzymes are depleted side of the organelle, whereas Golgi subfractions enriched for specific Golgi subcompartments. Our morphological results supports the assumption that glycosyltransferases are localized in specific Golgi subcompartments. The presence of immunolabel for galactosyltransferase in trans (inner maturing) Golgi cisterna that are positive for TPPase activity and are in accordance with a model that proposes a concerted action of both galactosyltransferase and nucleoside diphosphatase in chain elongation of maturing glycoproteins.

We thank Profs. H. Aebi and L. Orci for continuous interest and support, Mrs. Th. Mandel, Mrs. F. Fichard, and Mr. W. Seelentag for excellent technical assistance, and the helpful criticism of Dr. A. Tartakoff in reviewing the manuscript. The facilities for low-temperature embedding provided by Drs. E. Carlemalm and W. Villiger (Biocenter, University of Basel) are gratefully acknowledged.

This work was supported by grants 3.668.80 and 3.355.0.78 of the Swiss National Science Foundation and by the Sandoz Research Foundation, Basel, Switzerland.

Received for publication 20 July 1981, and in revised form 29 December 1981.

REFERENCES

1. Bennett, G., and D. O'Shaughnessy. 1981. The site of incorporation of sialic acid residues into glycoproteins and the subsequent fates of these molecules in autography after injection of [14C]N-acetylglucosamine. 1. Observations in hepatocytes. J. Cell Biol. 88:1-15.

2. Berger, E. G., Th. Mandel, and U. Schil. 1981. Immunohistochemical localization of galactosyltransferase in human fibroblasts and HeLa cells. J. Histochem. Cytochem. 29:364-373.

3. Bretz, R., H. Bretz, and G. E. Palade. 1980. Distribution of terminal glycosyltransferases in hepatic Golgi fractions. J. Cell Biol. 84:87-101.

4. Carlemalm, E., M. Garavito, and W. Villiger. Resin development for electron microscopy and an analysis of embedding at low temperature. J. Micrscope (Oxford) 1:16-18.

5. Ehrenreich, J. H., J. J. M. Bergeron, P. Siekevitz, and G. E. Palade. 1973. Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. J. Cell Biol. 59:945-72.

6. Elting, J. J., W. W. Chen, and W. J. Lennarz. 1980. Characterization of a glucosidase involved in an initial step in the processing of oligosaccharide chains. J. Biol. Chem. 255:2325-2331.

7. Faragher, M. G. 1978. Traffic of products and membranes through the Golgi complex. In Transport of Macromolecules in Cellular Systems. S. C. Silverstein, editor. Dahlem Konferenzen, Berlin. M1-1-362.

8. Fleischer, B., and S. Fleischer. 1970. Preparation and characterization of Golgi membranes from rat liver. Biochim. Biophys. Acta. 219:301-310.

9. Gerber, A. Ch., I. Kordowski, S. R. Wysa, and E. G. Berger. 1979. The charge heterogeneity of soluble human galactosyltransferases isolated from milk, amniotic fluid and malignant ascites. Eur. J. Biochem. 95:493-460.

10. Hartnas, N., and H. Schachter. 1980. Control of glycoprotein synthesis. Processing of asparagine-linked oligosaccharides by one or more rat liver Golgi α-N-acetylgalactosaminidase dependent on the prior action of UDP-N-acetylgalactosamine: α-N-acetylgalactosaminyltransferase and the subsequent fate of these molecules in autography after injection of [14C]N-acetylglucosamine. 2. Observations in hepatocytes. J. Cell Biol. 88:1-15.

11. Hino, Y., A. Asano, and R. Sato. 1978. Biochemical studies on rat liver Golgi apparatus. III. Subfractionation of fragmented Golgi apparatus by current-current distribution. J. Biochem. (Tokyo). 83:935-942.

12. Kellinberger, E., E. Carlemalm, W. Villiger, J. Roth, and R. M. Garavito. 1980. Low denaturation embedding for electron microscopy of thin sections. Chemische Werke Lowi GmbH, Waldkraiburg, FRG. 1-59.

13. Kuhn, N. J., and A. White. 1977. The role of nucleoside diphosphatase in an uridine nucleotide cycle associated with lactose synthetase in rat mammary gland. Biochim. J. 168:422-433.

14. Morré, D. J., J. Kartenbeck, and W. W. Franke. 1979. Membrane flow and interconversion between endomembranes. Biochim. Biophys. Acta. 559:71-152.

15. Morré, D. J., L. M. Merlin, and T. W. Keenan. 1969. Localization of glycosyltransferase activities in a Golgi apparatus-rich fraction isolated from rat liver. Biochem. Biophys. Res. Commun. 37:813-819.

16. Novikoff, A. B. 1963. Lysosomes in the physiology and pathology of cells: contributions of staining methods. In Ciba Foundation Symposium on Lysosomes A. V. S. de Reuck and M. P. Cameron, Churchill Ltd., London. 36-73.

The codistribution of galactosyltransferase with TPPase was directly demonstrated by a double-staining approach. The presence of immunolabel for galactosyltransferase in trans cisternae corresponding to the distribution of TPPase activity supports the assumption that glycosyltransferases are localized in specific Golgi subcompartments. Our morphological results confirm biochemical evidence obtained by Hino et al. (11) that galactosyltransferase activity is associated with TPPase in Golgi subfractions which probably correspond to the secretary (trans) side of the organelle, whereas Golgi subfractions enriched for several endoplasmic reticulum "marker" enzymes are depleted in glycosyltransferase activities. The close relationship between galactosyltransferase and TPPase strongly supports a model of glycosylation proposed by Kuhn and White (13) which we have extended as shown in Fig. 13. In this context it is of particular interest that Yamazaki et al. (30) demonstrated that nucleoside diphosphatase paralleled TPPase activity over the whole purification procedure applied to bovine liver microsomes and that both activities are manifested by a single enzyme. The membrane-association of galactosyltransferase has not been demonstrated by our immunocytochemical results due to the limited lateral resolution of the pAg technique. Evidence for it is based on detergent activation of the Golgi-membrane enzyme reported by numerous investigators (2, 3, 15). In conclusion, the data demonstrate the presence of galactosyltransferase in trans (inner maturing) Golgi cisternae that are positive for TPPase activity and are in accordance with a model that proposes a concerted action of both galactosyltransferase and nucleoside diphosphatase in chain elongation of maturing glycoproteins.

FIGURE 13 Schematic localization of both galactosyltransferase and TPPase (or nucleoside diphosphatase) in trans cisternae of the Golgi apparatus in HeLa cells. Codistribution of both enzymes supports a model of glycosylation proposed by Kuhn and White (13) for lactose biosynthesis in the mammary gland; On the basis of kinetic evidence, a carrier-mediated transport mechanism for glucose, UDP-galactose, and UMP through Golgi membranes and a diffusion barrier for UDP were postulated. Galactosyltransferase (GT) catalyzes the transfer of galactose (A) to glycoprotein acceptors containing GlcNAc (■) at the nonreducing end of the heteroglycan. This reaction generates free UDP, which is immediately hydrolyzed by UDPase (or TPPase). This prevents the build-up of inhibiting concentrations of UDP.
17. Novikoff, A. B., and S. Goldfischer. 1961. Nucleoside diphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. *Proc. Natl. Acad. Sci. U. S. A.* 47:802-810.

18. Orci, L., R. Montesano, P. Meda, F. Malaize-Lagae, D. Brown, A. Perrelet, and P. Vassalli. 1981. Heterogeneous distribution of flippin-cholesterol complexes across the cisternae of the Golgi apparatus. *Proc. Natl. Acad. Sci. U. S. A.* 78:293-297.

19. Poort, C. 1977. In situ localization of galactosyltransferase in surface mucous cells of the rat stomach. *J. Histochem. Cytochem.* 25:57-60.

20. Roth, J. 1981. The protein A-gold (pAg) technique. Qualitative and quantitative approach for antigen localization on thin sections. In *Immunocytochemistry Techniques and Applications*. G. R. Bullock and P. Petrusz, editors. Academic Press, Inc., London. In press.

21. Roth, J., M. Bendayan, E. Carlemalm, W. Villiger, and M. Garavito. 1981. Enhancement of structural preservation and immunocytochemical staining in low temperature embedded pancreatic tissue. *J. Histochem. Cytochem.* 29:663-671.

22. Roth, J., M. Bendayan, and L. Orci. 1978. Ultrastructural localization of intracellular antigens by the use of protein A-gold complex. *J. Histochem. Cytochem.* 26:1074-1081.

23. Roth, J., M. Ravazzola, M. Bendayan, and L. Orci. 1981. Application of the protein A-gold technique for electron microscopic demonstration of polypeptide hormones. *Endocrinology* 108:247-253.

24. Roth, J. B. Thorens, W. Huhniker, A. W. Horan, and L. Orci. 1981. Vitamin D dependent calcium binding protein: immunocytochemical localization in chick kidney. *Science* (Wash. D. C.) 214:197-199.

25. Rothman, J. E. 1981. The Golgi apparatus: Two organelles in tandem. *Science* (Wash. D. C.) 213:1212-1219.

26. Schachter, H., and S. Roseman, 1980. Mammalian glycosyltransferases. Their role in the synthesis and function of complex carbohydrates and glycolipids. In *The Biochemistry of Glycoproteins and Proteoglycans*. W. J. Lennartz, editor. Plenum Press, New York. 85-160.

27. Struck, D. K., and W. J. Lennartz. 1980. The function of saccharide-lipids in synthesis of glycoproteins. In *The Biochemistry of Glycoproteins and Proteoglycans*. W. J. Lennartz, editor. Plenum Press, New York. 35-83.

28. Tartakoff, A. M. 1980. The Golgi complex: crossroads for vesicular traffic. *Int. Rev. Exp. Pathol.* 22:227-251.

29. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U. S. A.* 76:4350-4354.

30. Yamazaki, M., and O. Hayaishi. 1968. Allosteric properties of nucleoside diphosphatase and its identity with TPPase. *J. Biol. Chem.* 243:2934-2942.