Synthesis and Turnover of Plasma-Membrane Proteins and Glycoproteins in a Neuroblastoma Cell Line

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A kinetic analysis of the appearance of ^{14}C -labelled proteins in the surface membranes isolated from exponentially growing neuroblastoma cells (N2a) showed that the total membrane proteins reached a steady-state specific radioactivity in 18–20 h. However, examination of individual protein bands resolved by sodium dodecyl sulphate–urea–polyacrylamide-gel electrophoresis illustrated that differences in the kinetics of specific surface-membrane proteins could be detected. Although most of the protein bands reached a steady-state specific radioactivity at a time similar to that for total membrane proteins, at least two bands (mol.wt. 180 000 and 130 000) attained the steady-state within 8–10 h. It was shown by the use of dual-labelling techniques that these two protein bands turned over in the surface membranes of neuroblastoma N2a cells at least 180 and 150% faster than the total membrane protein. These two proteins were glycosylated and located on the outer surface of the cells, since they were labelled with radioactive carbohydrates and readily removed by treatment of the intact neuroblastoma cell with proteinases.

The role of the limiting plasma membrane in the regulation of transport of cellular nutrients and products, as well as its putative role in regulating cell proliferation, has occupied a central position in biology. Alterations in plasma-membrane constituents during productive viral infection (Anshel, 1973) and viral transformation (Wickus & Robbins, 1973) have suggested that functional membrane modifications may be necessary for these processes. Subtle changes in cell-surface membrane composition or topography between normal and transformed mammalian cells, measured by lectin binding and cell agglutination, have been reported (Burger & Goldberg, 1967; Burger, 1971). Hynes (1973) demonstrated qualitative changes in the cell-surface protein composition of transformed cells.

The synthesis, assembly and degradation of plasma membranes themselves involves a dynamic series of events in which the membrane components appear to be in a constant metabolic flux, which is consistent with the mosaic model of membrane structure and biosynthesis (Arias *et al.*, 1969; Dehlinger & Schimke, 1971; Gurd & Evans, 1973). The synthesis and degradation of plasma-membrane constituents may actually proceed at about equal rates in growing and

stationary-phase cells (Warren & Glick, 1968). Although this would imply that cells continually degrade and replace all components of the plasma membrane, Graham *et al.* (1973) have suggested that the major constituents may preferentially increase during the G_1 phase of the growth cycle. In addition, reticulocytes appear to synthesize actively only two of their ten major membrane protein components (Lodish, 1973). Although these studies have provided information about membrane formation and modification, the kinetics of synthesis and turnover of different species of proteins and glycoproteins in the surface membrane of the eukaryotic cell and their possible functional roles is at present obscure.

Because of the lack of information available on plasma-membrane synthesis and turnover of neuroblastoma cells, we undertook the present study, which was designed to define kinetically the rates of these processes for individual membrane classes, as well as to determine the location of several of these proteins on the cell surface. Evidence of membrane structure (Shubert *et al.*, 1971; Truding *et al.*, 1974) and surface composition (Glick *et al.*, 1973; Akeson & Herschman, 1974) of neuroblastoma cells suggest that alterations in plasma membranes may accompany 'morphological differentiation'. To our knowledge, this is the first kinetic analysis of the metabolism of discrete plasma-membrane components and their possible relationship to surface-membrane structure.

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Materials and Methods

Incubation of neuroblastoma cells

Neuroblastoma cells (N2a), obtained from Dr. A. Telsar of Northwestern University Medical School, were propagated in monolayer culture at 37°C, under a CO₂+air (5:95) atmosphere, in humidified incubators. The cells were grown as a routine in 100mm plastic culture dishes with 15 ml of Dulbecco's Modified Eagle's Medium, supplemented with dextrose (3.5g/l), NaHCO₃ (3.7g/l), antibiotics (penicillin, 100 units/ml; streptomycin, 100 µg/ml; fungizone, 0.25 µg/ml), and 10% γ -globulin-free newborn calf serum. All cell-culture-medium components were purchased from Grand Island Biological Co. (Grand Island, N.Y., U.S.A.). The incorporation of radioactive precursors into neuroblastoma-cell membranes was measured by incubating the cells in complete medium as described above with the addition of the appropriate ¹⁴C- or ³H-labelled precursors. Plasma-membrane protein synthesis was measured with the addition of either 2.5 µCi of a ¹⁴C-labelled amino acid mixture (15 amino acids, 96–468 mCi/mmol)/ml or 8.0 µCi of a ³H-labelled amino acid mixture (15 amino acids, 3–58 Ci/mmol)/ml, which were purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.). Glycoprotein metabolism was measured by the addition of 8.0 µCi of either [6-³H]fucose or [6-³H]glucosamine (New England Nuclear Corp.). After incubation of the cells with the radioactive precursors for the time-periods indicated in the specific experiments, cells were harvested in 2.0ml of ice-cold 0.15M-NaCl by scraping with a rubber 'policeman'.

Plasma-membrane isolation

Plasma membranes from neuroblastoma cells were isolated by the Zn²⁺-ion method essentially as described by Warren & Glick (1969). Briefly, the cells harvested from tissue culture were washed twice with 0.15M-NaCl to remove traces of media and extraneous radioactivity. The cells were then centrifuged into a pellet at 200g for 5 min. The cell pellet of the second washing was resuspended in 5 ml of 0.15M-NaCl, mixed with 15 ml of 1mM-ZnCl₂ and maintained at room temperature with occasional mixing for 10 min. The cells were disrupted by homogenization with a motor-driven glass-Teflon homogenizer and the homogenate was made 20% (w/v) in sucrose. A preliminary membrane fractionation was performed by layering the homogenate over a 100ml discontinuous gradient containing 65, 40 and 35% unbuffered sucrose (2:1:1, by vol.). The gradient was centrifuged at 8000g for 10 min and the membrane band was recovered from the 65/40% sucrose interface. The membrane fraction was then diluted three-fold with water and the membranes were pelleted at 34000g for 10 min. The pellet was resuspended in

10ml of 35% sucrose, layered over a second discontinuous gradient containing 10ml each of 65, 55, 50, 45 and 40% sucrose and centrifuged at 90000g for 90 min. The membranes were recovered from the 65/55% and 55/50% interface boundaries, pooled, diluted to 60ml with water, and concentrated by pelleting the membranes at 90000g for 60 min. Finally, the pellet of purified plasma membrane was resuspended in 0.15M-NaCl. The protein content of the preparation was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as a protein standard, and the membranes were stored at -20°C at a concentration of about 2 mg of protein/ml.

The isolated membranes were relatively free of cytosol proteins, since we have measured by isotope dilution that less than 0.4% of the proteins in the final plasma-membrane preparations originate from the cell cytoplasm. In addition, examination of the final plasma-membrane preparations by electron microscopy illustrated that the samples were primarily composed of large membrane fragments with little or no contamination with cellular ribosomes, mitochondria or other particulate material.

When the specific radioactivity of the labelled membrane preparation was to be measured, samples of the isolated membrane material were precipitated with trichloroacetic acid (5%, w/v, final concn.). The precipitate was collected on a Millipore membrane filter, washed with 2×5 ml of 5% trichloroacetic acid, and the radioactivity was determined in the toluene-based scintillation fluid of White (1968).

Sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis

Membrane proteins from the purified plasma membranes were separated on sodium dodecyl sulphate/urea/polyacrylamide gels by the method of Fairbanks *et al.* (1971), except that 0.5M-urea was included in the gels and sample buffer. Samples (0.1 ml) containing 120 µg of membrane protein were layered over 12cm 5.6% polyacrylamide gels containing 1% sodium dodecyl sulphate and 0.5M-urea and electrophoresed at 5mA/gel for 18–20h.

Evaluation of gels. Membrane-protein bands were directly located by staining the gels overnight in a solution containing 0.025% Coomassie Blue stain, 25% (v/v) propan-2-ol and 10% (v/v) acetic acid. Before destaining, the gels were placed in a secondary staining mixture containing 0.0025% Coomassie Blue, 10% (v/v) propan-2-ol and 10% (v/v) acetic acid for 4h. Destaining was performed by placing the gels in 10% acetic acid for 48h with four fresh changes of 10% acetic acid. The protein bands resulting from this procedure were quantified by scanning the gels at 540nm with a Gilford spectrophotometer fitted with a linear transport and an automatic strip-chart recorder.

Electrophoretic profiles of the gels containing labelled proteins were obtained by freezing the gels in solid CO_2 /ethanol and slicing them into 1mm fractions with a BioRad Gel Divider (model 190). Each fraction was treated with 0.5ml of Nuclear-Chicago Solubilizer (Nuclear-Chicago, Chicago, Ill., U.S.A.) at 55°C for 2h (Arias *et al.*, 1969). Scintillation counting was performed by the addition of 10ml of toluene-based scintillation fluid (White, 1968) to each vial and counting for radioactivity in a Packard Tri-Carb liquid-scintillation counter (model 3310). Approximately 4cm of the lower end of the gels was discarded since we never detected stained protein nor radioactivity in this portion of the gels after electrophoresis.

Molecular-weight calibration of sodium dodecyl sulphate/urea gels. Molecular-weight determination of membrane proteins was approximated by comparing the electrophoretic mobilities with those of proteins of known molecular weight. γ -Globulin (160000), bovine serum albumin (69000) and chymotrypsinogen (25000) were used as standards.

Proteolytic-enzyme treatment of intact neuroblastoma cells

The effect of proteolysis on the plasma-membrane proteins of intact cells was tested by treating cells (preincubated with radioactive amino acids) with a 0.25% (v/v) solution of Viokase (Research Products Industries, Cary, Ill., U.S.A.) for 2 min at 37°C and immediately diluting the cells 100-fold with ice-cold 0.15M-NaCl. The cells were then washed with ice-cold 0.15M-NaCl and the plasma-membrane fraction was purified as described above.

Results

Protein composition of the plasma membrane

To determine the protein composition of the purified plasma membranes from exponentially growing neuroblastoma cells, membrane proteins were extracted and separated by sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis. Fig. 1 shows the E_{540} profile obtained by staining the gel with Coomassie Blue for protein. The profile was highly reproducible and shows a number of peaks of diverse molecular weights. By comparison with proteins of known molecular weight, the bands of plasma-membrane proteins move to positions in the gel corresponding to a molecular-weight range of from 30000 to well over 200000. It is possible that the large band at the top of the gel (mol.wt. about 300000) arises from aggregation of other membrane proteins; however, its presence was noted even when large amounts of reducing agent (2-mercaptoethanol) or EDTA were used in the preparation of the proteins for electrophoresis in 1% sodium dodecyl sulphate.

Kinetics of total membrane-protein labelling and of individual protein components

The kinetics of labelling of the total membrane protein are shown in Fig. 2. Exponentially growing neuroblastoma cells were incubated in complete media containing a ^{14}C -labelled amino acid mixture, and at the various times indicated the cells were washed free of label and the plasma-membrane fraction was isolated as described above. The specific radioactivity of the membrane was determined in samples of the purified membrane fraction as described in the Materials and Methods section. Within 18–20h of labelling the bulk of the plasma-membrane protein attained a steady-state specific radioactivity.

As a comparison with the total membrane protein, the kinetics of labelling of discrete species of plasma-membrane proteins were determined by subjecting samples of the membranes labelled for various times to sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis and observing the increase in radioactivity associated with different molecular-weight protein components (Figs. 3a–3e). The profiles indicate that the specific radioactivity of many of the protein components of the membrane increases at approximately the same rate. This suggests that these

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of plasma-membrane proteins

Neuroblastoma cells were grown in 100mm Falcon plastic culture dishes. Cells were scraped from the plastic surface and washed with $2 \times 25\text{ml}$ of 0.15M-NaCl. Plasma membrane fractions were prepared by the Zn^{2+} method, the membrane protein was solubilized with 1% sodium dodecyl sulphate and 120 μg of protein was electrophoresed on sodium dodecyl sulphate/urea gels as described in the Materials and Methods section. The gels were stained for protein and scanned for E_{540} as described. The molecular-weight axis is provided for reference.

Fig. 2. Kinetics of incorporation of ^{14}C -labelled amino acids into plasma-membrane protein

Neuroblastoma cells were grown in the presence of 2.5 μCi of a ^{14}C -labelled amino acid mixture/ml. At 4, 8, 12, 18 and 24 h of labelling cultures were harvested by scraping the cells into ice-cold 0.15M-NaCl and mixed with unlabelled cells used as a carrier. Plasma membranes were prepared and the specific radioactivity of the membrane protein was determined as described. The results are expressed as c.p.m./mg of trichloroacetic acid-insoluble membrane protein.

proteins of the membrane are being actively synthesized in growing neuroblastoma cells. However, a closer analysis of these data indicated that at least two species of membrane protein reached steady-state specific radioactivities much earlier than the other components. When the radioactivity in the profiles in Fig. 3 is expressed by plotting the c.p.m. in the peak fractions versus the duration of the labelling period, a family of curves is generated as shown in Fig. 4. The kinetics of labelling of five representative protein bands are shown in Fig. 4(a). Most of the bands appeared to reach their maximum specific radioactivity within 12–18 h. At least one of these protein components appeared to be labelled linearly with time up to 24 h. In contrast with the kinetics of labelling found for most of the individual membrane proteins, and with the kinetics of the overall membrane-protein labelling shown in Fig. 2, two protein bands (mol. wts. 180 000 and 130 000) were more rapidly labelled and reached steady-state specific radioactivities in 8 h (Fig. 4b). It must be emphasized that the curves shown in Figs. 4(a) and 4(b) represent a true increase in

Fig. 3. Sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis of ^{14}C -labelled plasma-membrane protein

Samples of the plasma-membrane preparations labelled with ^{14}C -labelled amino acids (see legend to Fig. 2) were subjected to sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis as described in the Materials and Methods section. (a)–(e) Radioisotopic profiles of 120 μg of plasma-membrane protein from cells grown in the presence of ^{14}C -labelled amino acids for 4 (a), 8 (b), 12 (c), 18 (d) and 24 h (e). Each gel fraction was 1 mm, as described in the Materials and Methods section.

Fig. 4. Kinetics of incorporation of representative protein peaks into the plasma-membrane of neuroblastoma cells

The experimental details are described in the legend to Fig. 3. The data represents the radioactivity (c.p.m.) in the peak fractions from the profiles in Fig. 3 plotted against the time of labelling. (a) Kinetics for membrane components of mol.wt. 55000 (▽), 65000 (□), 110000 (Δ), 250000 (○) and 300000 (●). (b) Kinetics of incorporation for the components of mol.wt. 130000 (■) and 180000 (▲).

specific radioactivity, since equal amounts of protein were applied to the gels and the protein profiles were highly reproducible.

Turnover of discrete plasma-membrane protein components

It is possible that the two protein components of the plasma membrane that are synthesized and incorporated into membranes the most rapidly (mol.wts. 180000 and 130000) are turning over more frequently than the other components. To test this possibility the relative rates of turnover of individual membrane components were measured by a double-label technique. Rapidly growing neuroblastoma cells were incubated with culture media supplemented with a ^{14}C -labelled amino acid mixture. At the time indicated the cells were washed free of label and reincubated with media containing a ^3H -labelled amino acid mixture. Plasma membranes were prepared as described above, and the membrane proteins were extracted with 1% sodium dodecyl sulphate and separated on sodium dodecyl sulphate/urea/polyacrylamide gels. Fig. 5 shows the $^3\text{H}/^{14}\text{C}$ ratio obtained by slicing the gel and counting each fraction for both radioactive isotopes. Under the conditions of this experiment the protein components that show the highest ratio of radioactivity counts have been incorporated, removed and reincorporated into the

Fig. 5. Double-labelling of neuroblastoma plasma membranes

Neuroblastoma cells were grown in complete medium supplemented with a ^{14}C -labelled amino acid mixture ($2.5 \mu\text{Ci/ml}$) for 24 h. The cells were then rinsed with fresh medium and reincubated with complete medium supplemented with a ^3H -labelled amino acid mixture ($8 \mu\text{Ci/ml}$) for 8 h. The cells were then harvested and the plasma membranes isolated as described. Plasma-membrane proteins were solubilized in 1% sodium dodecyl sulphate and separated by sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis as described in the Materials and Methods section. The gels were sliced and the ^{14}C (●) and ^3H (○) radioactivities in each 1 mm slice were determined (a). (b) Ratio of $^3\text{H}/^{14}\text{C}$ radioactivity for each fraction. ———, 99% tolerance limits of variability, which was determined in separate control experiments as described by Arias *et al.* (1969). In cells simultaneously incubated with ^3H - and ^{14}C -labelled amino acid mixtures, no bands significantly deviated from the normalized ratio ($P = 0.01$).

membrane the most frequently during the ^3H chase. Of particular interest is the fact that the protein peaks that show the highest $^3\text{H}/^{14}\text{C}$ ratios correspond to the components that are labelled to steady-state values in the membrane within 8 h (Figs. 3a–3e and 4b). These high-molecular-weight components of the membrane appear to be turning over 180 and 150% faster than the bulk of the membrane protein. The possibility that the increased ratio of $^3\text{H}/^{14}\text{C}$ in these two bands was an artifact of a preferential incorporation of specific amino acids in the mixtures was

Fig. 6. Glycosylation of plasma-membrane protein

Neuroblastoma cells were grown in complete media with $8 \mu\text{Ci}$ of either $[6\text{-}^3\text{H}]\text{fucose}$ or $[6\text{-}^3\text{H}]\text{glucosamine/ml}$ (specific radioactivity of each was 15 Ci/mmol) for 24 h. Plasma membranes were purified from the cells and the proteins extracted with sodium dodecyl sulphate and separated on sodium dodecyl sulphate/urea/polyacrylamide gels as described. (a) ^3H profiles for fucose (-----) and glucosamine (—); (b) molar ratio of the incorporated radioactive fucose/glucosamine in each 1 mm fraction.

eliminated by control experiments. When neuroblastoma cells were incubated simultaneously with ^3H - and ^{14}C -labelled amino acid mixtures, the cell membranes isolated and the components resolved by sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis, no peaks significantly deviated from the average $^3\text{H}/^{14}\text{C}$ ratio ($P = 0.01$). By observing the decay of radioactivity from labelled membrane proteins we estimated that approx. 10% of the total protein turned over per generation (results not shown) in exponentially growing neuroblastoma cells. This value closely agrees with that of Warren & Glick (1969). However, this is surely an underestimate, since reutilization of radioactive precursors cannot be avoided under these experimental conditions.

Glycosylation of plasma-membrane protein

Since carbohydrate moieties comprise a significant proportion of the plasma-membrane material from a number of cell types, primarily as glycolipids (Bretscher, 1973; Yogeeswaran *et al.*, 1973) and glycoproteins (Bretscher, 1973; Atkinson & Summers,

1971; Hunt & Brown, 1974), we sought to determine which protein components of the neuroblastoma membrane were associated with carbohydrates. To determine the presence of glycoprotein the cultures were incubated for 24 h in complete media supplemented with either $[^3\text{H}]\text{fucose}$ or $[^3\text{H}]\text{glucosamine}$ as described in the Materials and Methods section. The plasma-membrane-protein profiles after electrophoresis are shown in Fig. 6(a). The profiles show that there is an abundance of carbohydrate-containing molecules that co-electrophorese with plasma-membrane proteins. There appeared to be at least ten peaks of radioactivity when cells were labelled with either fucose or glucosamine. Other studies (Bosmann *et al.*, 1969; Atkinson & Summers, 1971) have indicated that fucose is a rather specific precursor for plasma-membrane glycoproteins and that 70–90% of the fucose found in HeLa cells is localized in the outer membrane. Glucosamine is thought to be somewhat less specific for the plasma membrane (Atkinson & Summers, 1971).

Effect of proteolysis on plasma-membrane proteins

Studies have shown that mild proteolytic treatment of normal cells in culture can induce a transient stimulation of cell division (Burger, 1970). This stimulation may occur by modulating the intracellular cyclic AMP concentration (Burger *et al.*, 1972). It has been demonstrated that proteinase-sensitive components of the surface of normal cells are deleted from the surface of their transformed counterparts (Hynes, 1973; Gahmberg & Hakomori, 1973).

To ascertain which, if any, of the components of the neuroblastoma plasma membrane are sensitive to proteolytic enzymes, we treated intact cells grown for 24 h in ^{14}C -labelled amino acid-supplemented medium with Viokase (a mixture of proteolytic enzymes used as a routine to remove monolayer cells from culture surfaces). After proteinase treatment the cells were repeatedly washed and the plasma membranes were purified and the proteins separated on sodium dodecyl sulphate/urea/polyacrylamide gels as described above. The effects of Viokase treatment of intact cells are shown in Fig. 7. Marked alterations in the normal profile of radioactivity from untreated controls were evident after enzyme treatment. Viokase produced its most striking effect on the lower-molecular-weight classes of protein. Similar effects were also observed by the treatment of intact cells with trypsin (results not shown). The membrane-protein species most susceptible to proteolytic attack are those of about 40000, 60000, 70000, 130000 and 180000 mol.wt. It is likely that the components of the surface membrane that are proteinase-sensitive are located on or exposed to the outer surface of the cell. The possibility that proteolysis removes intramembraneous protein structures of those inside the

Fig. 7. Effect of Viokase treatment of whole cells on plasma-membrane proteins

Neuroblastoma cells were grown for 24h in complete medium supplemented with $2.5 \mu\text{Ci}$ of a ^{14}C -labelled amino acid mixture/ml. The cells were harvested from the culture dishes and treated with Viokase (----) or left untreated (—). The membrane proteins were separated by sodium dodecyl sulphate/urea/polyacrylamide-gel electrophoresis of samples of the purified plasma-membrane fractions as described. The molecular-weight axis is provided for reference.

cell is considered unlikely, since the cells remain intact during enzyme treatment and are viable when re-cultured.

Discussion

By measuring the kinetics of incorporation of labelled amino acids into the proteins of plasma-membrane structures, we have shown that discrete components of the membrane are assembled at different rates. Many of the individual protein components are incorporated into the surface membranes at rates similar to that of the overall protein, reaching steady-state specific radioactivities at about 18h of exposure to radioactive amino acids. At least two species of membrane protein are synthesized much more rapidly, and reach steady-state values in about 8h. These two components are of relatively high molecular weight (180000 and 130000), but together comprise only a small fraction of the total membrane protein (Figs. 4 and 5).

We have further shown that the rapid rate of labelling of these two components is due to a very high rate of turnover in the membrane. Experiments using double-labelling techniques indicate that the 180000- and 130000-mol.wt. components turn over 180 and 150% faster respectively than does the bulk of the

membrane protein. This estimate of turnover is most probably an underestimate, since reutilization of ^{14}C -labelled amino acids could not be completely avoided. It has been shown that proteins from intracellular membranes (endoplasmic reticulum and zymogen granules) of pancreatic acinar cells were different in their turnover properties (Meldolesi, 1974), substantiating that the metabolic regulation of protein assembly into membranous structures may be different for individual components. It is conceivable that the glycosylated membrane proteins that are turning over at the highest frequency are actually extracellular secretory products in transit through the surface membrane.

In membrane preparations in which carbohydrates were labelled by incubating the cells in the presence of ^3H fucose or ^3H glucosamine we find large amounts of this radioactivity co-electrophoresing with protein. In contrast with reticulocytes (Bretscher, 1973), HeLa cells (Atkinson & Summers, 1971) and mouse L cells (Hunt & Brown, 1974), in which a few glycoproteins predominate in the membrane, neuroblastoma cells appear to have numerous species of glycoproteins located in their surface membranes. It would be of great interest to determine if the specificity or extent of glycosylation of membrane proteins may play a role in the metabolic regulation of protein turnover or conformation.

Finally we have shown that several of the protein components of the plasma membrane are located on, or exposed to, the surface of the cell by virtue of their susceptibility to proteolytic enzymes (Fig. 7). Treatment of whole cells with Viokase removes several protein components of the plasma membrane, most notably the 180000- and 130000-mol.wt. species. These two high-molecular-weight proteins are identical with the components that were shown to be turning over in the membrane at the highest rate. Perhaps the susceptibility to surface proteolytic attack is related to the increased rates of turnover of these membrane proteins. It has been suggested that transformed cells produce proteinases (Bosmann, 1972; Unkeless *et al.*, 1973; Ossowski *et al.*, 1973) and that these types of substances are able to stimulate cell division (Burger, 1970; Burger *et al.*, 1972; Rubin, 1970). In addition, mesenchymal growth factor (Attardi *et al.*, 1967) and nerve growth factor (Greene *et al.*, 1968) possess proteinase activity. Recent experiments demonstrated that proteinase-sensitive surface glycoproteins differ in transformed and normal cells (Hynes, 1973; Gahmberg & Hakomori, 1973). Perhaps the growth potential of cells may be related to their surface architecture (Burger & Goldberg, 1967; Burger, 1971), which in turn may be altered by external stimuli such as proteolysis. Indeed, transformed cells can be made to revert to normal growth behaviour by exposing them to inhibitors of proteolytic enzymes (Schnebli &

Burger, 1972). However, some caution must be used in the interpretation of results from studies that include proteinase inhibitors, since these agents disrupt cellular protein synthesis (Chou *et al.*, 1974). Despite these limitations, it should be possible to study the relationship of the turnover of the proteins and glycoproteins in the surface membranes of neuroblastoma cells and the cellular growth kinetics and morphological state (Akeson & Herschman, 1974; Truding *et al.*, 1974).

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