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The role of eucaryotic cell surface glycoconjugates in DNA replication

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Abstract. Multiple forms of ricin have been isolated from castor bean seeds. Two forms, ricin-1 and ricin-2, differ in their isoelectric pI values and toxicity towards IMR-32 cells. Inhibition of IMR-32 DNA polymerase α_2 is more pronounced with ricin-1 (65%) than with ricin-2 (10%). Ricin B chain (pI = 5.2) isolated from ricin-1 binds to IMR-32 cell surfaces as well as inhibits DNA polymerase α_2 activity when studied *in vitro.* The presence of galβ-linked glycoconjugates near the active site of IMR-32 DNA polymerase α_2 has been proposed. Replication modulators which bind to the glycose portion of the enzymes involved in the replication system may need a mandatory binding to cell surface glycoconjugates for their activity.

Keywords. Cell surface; DNA biosynthesis; DNA polymerase α; glycoconjugates; IMR-32 cells; ricin b; neuroblastoma cells.

Introduction

The complex biochemical steps that lead to the control of chromosomal DNA replication during differentiation of eucaryotic cells are not yet understood. To clarify the mechanism of expression of various properties of differentiated neurons, many studies have been performed with mouse neuroblastoma cells (Augusti-Tocco and Sato, 1969; Amano *et al.,* 1972; Prasad and Sinha, 1978) or neuroblastoma *x* glioma hybrid cells (Nelson *et al.,* 1976; McGee *et al.,* 1978). These cells are induced to produce neurites in the absence of serum (Amano *et al.,* 1972; Basu *et al.,* 1976a; Prasad and Sinha, 1978 or in the presence of inhibitors of 3',5'-cyclic AMP (cAMP) phosphodiesterase (Basu *et al.,* 1976a;Moskal, 1977; Prasad and Sinha, 1978) or DNA biosynthesis (Schubert and Jacob, 1970; Moskal, 1977; Bhattacharya and Basu, 1978). Many factors appear to be involved in this process. During differentiation of neuronal (Basu *et al.,* 1976a; Prasad and Sinha, 1978) or non-neuronal cells (*e.g.* CHO cells, Hsie and Puck, 1971), cAMP seems to play an important but as yet uncharacterized role (Prasad and Sinha, 1978). Phosphorylation (Prasad and Sinha, 1978) and acetylation (Levy *et al.,* 1975; Ruben *et al.,* 1976; Marks *et al.,* 1978; Vidali *et al.,* 1978) of specific histones have been suggested as possible mechanisms involved in the arreast of cell division during differentiation of Friend virus-transformed murine erythroleukemia cells induced by dimethylsulfoxide or hexamethylenebisacetamide.

Alternatively, modification of the cell surface glycoconjugates may serve to increase or decrease the transport of serum nutrients or growth factors into the cell, leading eventually to re-initiation or "shutting off" of the cell cycle, as suggested by Holly (1972). Perhaps proteins synthesized inside the cytoplasm (endogenous)

or coming from outside the cell (exogenus) through a specific transport system present on the cell surface may play direct roles in DNA replication.

CHaracterization of galactose-containing glycoconjugates on neuroblastoma cell surfaces

Knowledge of the chemistry and biosynthesis of glycoconjugates present on neuroblastoma cell surfaces is still in its infancy. Recent studies from different laboratories (Rosenfeldor *et al.,*1977;Hakomori *et al.,*1980; Basu *et al.,*1980) have suggested that the distribution of glycoconjugates on tumor cell surfaces differs from that of normal cells. We thought in the early 1970's that the evaluation of such data would be facilitated by a systematic study of the phylogenetic distribution of the enzymes involved in the synthesis of cell-surface glycoconjugates. During the past ten years we have collected information about the relative activities of glycosyltransferases present in neuroblastoma cells of primate (human IMR-32) and nonprimate (mouse N-18) origins (Prasad and Hsie, 1971; Moskal *et al.,* 1974; Basu *et al.,* 1976a; Presper *et al.,* 1978). We have established the enzymatic pathway for the biosynthesis of the fucose-free B-active pentaglycosylceramide $nLcOse_5$ Cer (Galαl-3Galβl-4GlcNacβl-3Galβl-4Glc-Cer; figure 1) in both mouse N-18 and human IMR-32 neuroblastoma cells. In each case, synthesis

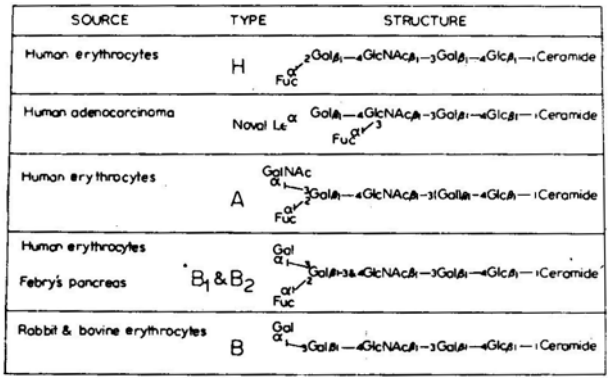

Figure 1. Structures ofblood group active glycosphingolipids.

takes place by stepwise elongation of the oligosaccharide chain by a specific glycosyltransferase (figure 2). Expression of an (α1 -2) fucosyltransferase required for the synthesis of terminal fucose-containing blood group-related glycosphingolipids (Basu *et al.,* 1975) appears to be confined to cultured cells of primate origin (Presper *et al.,* 1978).

Using specific lectins from *Bandeiraea simplicifolia* and *Ricinus communis,* we have established that these B-specific glycosphingolipids are exposed on the cell surfaces of both mouse and human neuroblastoma cells (Prasad and Hsie, 1971; Basu *et al.,*1976a, b) . Recently, using [^{125}I] -ricin, we have proved that both types of cells contain receptor sites which bind the ricin *B* subunit. On the basis of inhibition studies with methyl-β-D-galactopyranoside (Bhattacharya *et al.,* 1980), these receptor sites appear to be glycoconjugates containing terminal β-linked galactose. It is important to note that these cells contain high activities of UDP-galactose: $LcOse_3$ Cer (β1-4) galactosyltransferase (Basu and Basu, 1972; Presper *et al.,*

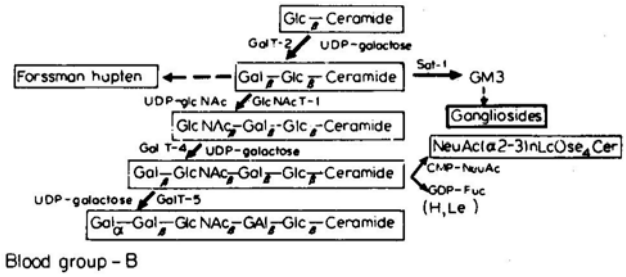

Figure 2. Proposed pathway for biosynthesis of blood group related glycosphingolipids.

1978), which catalyzes the synthesis of $nLcOse_4$ Cer (Galβl-4GlcNAcβl-3Galβl-4Glc-Cer; figure 2), an important intermediate of many blood group-active glycosphingolipids. Recently, Sundsmo and Hakomori (1976) have identified this glycosphingolipid as a potential tumor-associated antigen of polyoma-transformed NIL (NIL_{py}) cell surfaces.

Although specific glycosphingolipids have been identified on the cell surfaces of various chemically and virally transformed cells, their biological roles are not yet understood. Treatment of mouse neuroblastoma cells with the differentiating agent dibutyryl cyclin AMP (1 mM) reduces the binding of [^{125}I]-labelled *Bandeiraea simplicifolia* lectin (which recognizecs terminal α-linked galactose-containing glycoconjugates) to 30% of control values, suggesting a relationship between differentiation and cell surface composition. This is further supported by the findings of Nicolson *et al.* (1975), who demonstrated that an SV40-transformed 3 *T*3 cell line was more sensitive to the toxic effects of ricin than untransformed 3 *T*3 cells.

Lectin-induced inhibition of DNA synthesis

Ricin, a highly toxic glycoprotein isolated from castor beans (*Ricinus communis*), is a strong inhibitor of protein biosynthesis (Lin *et al.,*1970; Olsnes and Pihl, 1973; Hedblom *et al.,* 1978). It also in hibits [^{3}H] -thymidine incorporation into chromatin DNA of tumor cells (Lin *et al.,*1971). Previous studies on growth control and toxicity of ricin in cultured neuronal cells (Gonatas *et al.,*1975) suggest receptor-mediated endocytosis of intact ricin (M_r , 64, 000). Inhibition of protein biosynthesis in cell-free systems in the presence of the ricin *A* subunit has been reported by several laboratories, whereas the *B* subunit was generally assumed to have no function other than binding to the cell surface.

Early investigations in our laboratory on the effects of various lectins and toxins on the incorporation of [^{3}H] -thymidine into the DNA of human IMR-32 (Bhattacharya *et al.,* 1979) and mouse N-18 (unpublished) neuroblastoma clones revealed that total cellular DNA synthesis was reduced by concanavalin A and ricin. The failure of added serum to reverse the ricin-mediated inhibition of DNA synthesis suggested two possible mechanisms for its action:either ricin, by combining with the cell surface (Nicolson *et al.,* 1975), influenced the ability of the cell to respond to agents that initiate DNA synthesis, or it entered the cell (Gonatas, 1980) and directly inactivated one or more components of the DNA replication complex. The latter hypothesis was supported by our demonstration that ricin specifically inhibited human neuroblastoma DNA polymerase á activity *in vitro,*

while DNA polymerase β activity was unaffected (Bhattacharya *et al.,* 1979).

The existence of multiple forms of ricin has been reported (Olsnes and Pihl, 1973; Hedblom *et al.,* 1978). We have observed that differential toxicities toward cultured neuroblastoma IMR-32 cells among these multiple forms are due to differences in their *B* subunits. Recently, we have isolated different *B* chains with characteristics iso-electric points, and we are now studying their effects on the activities of multiple forms of DNA polymerase α.

Multiple forms of DNA polymerase α from human neuroblastoma IMR-32 cells

Multiple forms of DNA polymerase α have been identified in animal tissues (Momparler *et al.,* 1973; Hesslewood *et al.,* 1978; Yoshida *et al.,* 1974), solid tumors (Matsukage *et al.,* 1974; Chen *et al.,* 1979), and cultured tumor cells (Tanabe and Takahashi, 1976; Bieri-Bonniot and Schuerch, 1978; Ono *et al.,* 1979). Whether these forms have different primary structures, or whether they arise through modification of a common parental form is not yet known. Wilson and his co-workers (Chen *et al.,* 1979) recently purified to homogeneity from the soluble protein fraction of mouse myeloma two species of DNA polymerase α which consists of non-identical subunits with apparent molecular weights of 47,000 and 54,000. Tryptic peptide mapping of these two polymerases failed to show any sequence homology between the two subunits, although the intact enzymes share similar subunit molecular weights, native molecular weights, and catalytic properties. In contrast, Johnston and co-workers (Hesslewood *et al.,* 1978) showed that mild urea treatment of one form of calf thymus DNA polymerase α (*A* form, 8 S) generated a second form of the enzyme (*C* form, 7.3 S). They concluded that the *C* form was a single polypeptide and that the *A* enzyme contained an additional subunit of molecular weight 50,000-70,000. Whether the urea treatment dissociated a true subunit or a pre-existing proteolytic fragment is unclear.

Recently, we have reported (Bhattacharya *et al.,*1980,1981,1982) the isolation and characterization of three forms (α_1, α_2, α_3) of DNA polymerase α from IMR-32 human neuroblastoma cells. These forms display differential template specificities and heat sensitivities. The α_1 form very efficiently utilizes poly $(dA) \cdot (dT)_{12-18}$ poly (*dA-dT*), and activated calf thymus DNA as template-primers, while no activity could be detected using poly $(rA) \cdot (dT)_{12-18}$ DNA polymerase α_2 appears to utilize the poly $(rA)\ (dT)_{12-18}$ template-primer to a small but significant extent in addition to incorporating high levels of [^{3}H]-labelled deoxynucleotides into those templates preferred by the α_1 form. DNA polymerase α_3 exhibits maximal activity with poly $(dA)\ (dT)_{12-18}$ and poly (*dA-dT*); it also uses both activated calf thymus DNA and poly $(rA)\ (dT)_{12-18}$ but with considerably lower efficiency than forms α_1, and α_2.

These three forms are differentially inhibited by the ricin *B* subunit and by a nonhistone chromatin protein isolated from rat liver (Bhattacharya *et al.,* 1978, 1981). These findings suggest that the multiple forms may have different physiological functions in the replication of DNA in IMR-32 cells. This conclusion is

supported by the observation of changes in the levels of two forms of DNA polymerase α (*P*-I and *P*-II) during the HeLa cell cycle made by Ono *et al.* (1979) *P*-I activity increases between the G_1 and *S* phases, reaches a maximum at mid-*S* phase, and then declines rapidly. *P*-II activity is fairly constant throughout the cycle. The decrease in *P*-I activity may be due either to the appearance of an inhibitor or to decreased synthesis of the enzyme.

Conclusion

The overall objective of our research is to understand the complex biochemical events that lead to the initiation and termination of DNA replication in neuroblastoma cells during differentiation. Perhaps proteins synthesized in the cytoplasm (endogenous) or coming from outside the cell (exogenous) after binding on the cell surface may play direct roles in the control of DNA synthesis. Our recent studies on the inhibition of DNA polymerase α by an acidic nonhistone chromatin protein (NHCP) (Bhattacharya *et al.,* 1978,1981) and by ricin will serve as model systems for endogenous and exogenous effector proteins, respectively. The acidic protein (NHCP; M_r , 23,000) isolated from a mixture of rat liver chromatin proteins inhibits IMR-32 DNA Polymerases α_2 and α_3 by 85% and 83%, respectively. The nature of inhibition by this endogenous protein is a subject of our current investigation. Ricin is an ideal model for an exogenous protein which controls DNA synthesis. It binds specifically with terminal β-linked galactose-containing glycoconjugates, which are found in a wide variety of cell surfaces. Also, it specifically inhibits DNA polymerase α (Bhattacharya and Basu, 1982) isolated from human neuroblastoma IMR-32 cells. A similar reduction of neuroblastoma DNA polymerase α activity was observed (Bhattacharya and Basu, 1978) by treatment of mouse *N*-18 neuroblastoma cells with the differentiating agent 5-bromodeoxyuridine (BrdUrd). The steps that result in the reduction of chromosomal DNA replication in the presence of BrdUrd are not yet known. On the basis of our inhibition studies with ricin we propose a model for the control of DNA replication by an exogenous protein (figure 3) . It appears that cell-surface glycolconjugates may play an important role in recognition of such exogenous proteins.

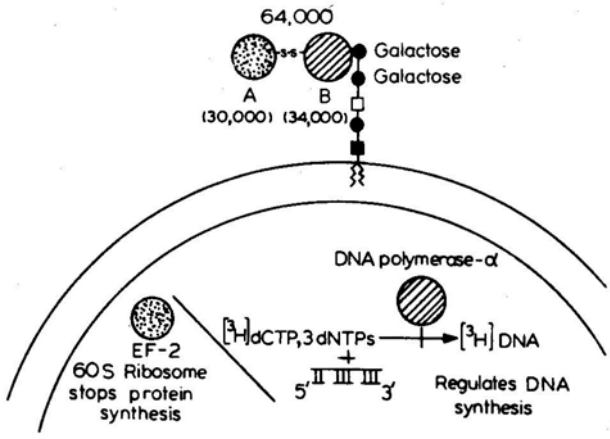

Figure 3. Proposed mechanism of ricin toxin action.

The expression of a specific cell-surface glycosphingolipid or glycoprotein during differentiation or transformation could therefore be a crucial step (figure 4) in the control of DNA replication by an exogenous protein.

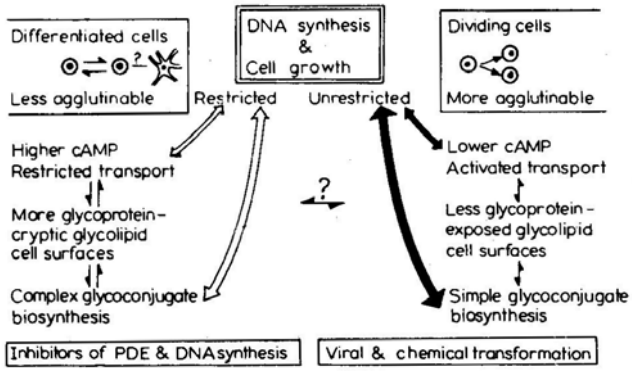

Figure 4. Interrelationship between cell growth and cell surfaces.

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