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Sialyltransferase activity in FR3T3 cells transformed with *ras* oncogene: decreased CMP-Neu5Ac:Galβ1-3GalNAc α-2,3-sialyltransferase

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We have investigated the activity of CMP-Neu5Ac:Galβ1-3GalNAc α-2,3-sialyltransferase (EC 2.4.99.4) in FR3T3 cells transformed by the Ha-*ras* oncogene in which we have previously demonstrated the higher expression of the β-galactosidase α-2,6-sialyltransferase (EC 2.4.99.1) [21].We demonstrate that the presence of the activated *ras* gene decreases the activity of this specific α-2,3-sialyltransferase fourfold. According to the kinetic parameters and to mixing experiments, we can assume that this decreased enzymatic activity reflects a decrease in the number of active *O*-glycan α-2,3-sialyltransferase polypeptides in *ras*-transformed cells. However, no change in the binding of Peanut agglutinin was observed on the cell surface of *ras*-transformed FR3T3 suggesting that no change in the sialylation of *O*-glycan core 1 appeared in these cells, although the activity of the α-2,3-sialyltransferase was decreased.

Keywords: c-Ha-*ras* oncogene, CMP-Neu5Ac:Galβ1-3GalNAc α-2,3-sialyltransferase, FR3T3 cells

Abbreviations: α-2,3-ST(*O*), CMP-Neu5Ac:Galβ1-3GalNAc-*R* α-2,3-sialyltransferase; α-2,3-ST(*N*/*O*), CMP-Neu5Ac:Galβ1-3/4GlcNAc-*R* α-2,3-sialyltransferase; α-2,6-ST(*N*), CMP-Neu5Ac:Galβ1-4GlcNAc-*R* α-2,6-sialyltransferase; α-2,6-ST(*O*)I, CMP-Neu5Ac:*R*-GalNAc(α1-*O*)Ser α-2,6-sialyltransferase; α-2,6-ST(*O*)II, CMP-Neu5Ac:Neu5Acα2-3Galβ1-3GalNAc-*R* α-2,6-sialyltransferase; ASFet, asialofetuin; FR3T3, Fisher rat fibroblast; FR*ras*, Ha-*ras*-transfected FR3T3 fibroblasts; NaCl/P_i, sodium phosphate 10 mM, NaCl 0.15 M, pH 7.4, buffer; *p*Np, *p*-nitrophenol.

Introduction

Alterations in cell surface carbohydrates is one of the most striking changes occurring in malignant cells, and the increased branching and the high level of sialylation have been associated with the metastatic capacity of tumour cells [1–8]. These changes in cell surface carbohydrates are supported by the deregulation of some specific glycosyltransferases. For example, the increased activity of the *N*-acetylglucosaminyltransferase V, the key enzyme in the synthesis of highly branched *N*-linked glycans, has been described and then associated with the invasive potential of transformed cells [9–11]. More recently, increased β1-6GlcNAc branching was demonstrated for both *N*- and *O*-linked glycans in experimental models of malignancy, and the role of this branching on the subsequent addition of poly-*N*-acetyllactosamine chains was demonstrated [12].

The sialylation of membrane glycoproteins occurs in the Golgi apparatus through sialyltransferases highly specific for the structure of the underlying acceptor [13]. Little is known about the regulation of the expression of the different sialyltransferases acting on *N*- and *O*-linked glycans but recent reports seem to indicate that these enzymes are partially, if not mainly, regulated at the transcriptional level. The CMP-Neu5Ac:Galβ1-4GlcNAc-*R* α-2.6-sialyltransferase (EC 2.4.99.1, α-2,6-ST(*N*)) gene produces multiple transcripts which are controlled by separate promoters and present striking tissue specific expression [14–17]. Moreover, the transcriptional level of this sialyltransferase in hepatocytes is controlled by several liver-enriched transcription factors [18] and by dexamethasone via the glucocorticoid receptor pathway [19, 20].

To date, it appears increasingly clear that the higher sialylation of membrane glycoproteins of transformed cells is due to a relative increase in some specific sialyltransferase activities rather than that of all the sialyltransferases present in these cells. For example, it was recently demonstrated that the activity of the α-2,6-ST(*N*) is increased in

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ras-transformed cells while the activity of the CMP-Neu5Ac:Galβ1-3/4GlcNAc-*R*, α-2,3-sialyltransferase (EC 2.4.99.6, α-2,3-ST(*N*/*O*)) remained unchanged [21–23]. This increased activity is the consequence of a higher level of transcription of the gene coding for this enzyme and leads to a higher amount of Neu5Acα2-6Gal-*R* sequences at the cell surface of *ras*-transformed cells [21]. The sialylation of *O*-linked chains is also modified in transformed cells. Indeed, increased sialylation of granulocyte membranes has been described in chronic myelogenous leukemia and correlated with an increased activity of an α-2,3-sialyltransferase with a strict specificity for *O*-linked glycan core 1, Gal(β1-3)-GalNAc(α1-*O*)Ser [24, 25]. This enzyme, which has been purified from porcine submaxillary gland [26, 27] and from human placenta [28], specifically sialylates galactose residues of the Gal(β1-3)GalNAc-*R* sequence via an α-2,3 linkage but cannot synthesize the Neu5Acα2-3Galβ1-4GlcNAc-*R* product [13].

In this report we investigate the activity of this enzyme in rat fibroblast (FR3T3 cells) transformed by the c-Ha-*ras* oncogene and we demonstrate that the expression of the activated *ras* gene decreases the activity of the CMP-Neu5Ac:Galβ1-3GalNAc-*R* α-2,3-sialyltransferase (EC 2.4.99.4, α-2,3-ST(*O*)) concomitantly with the increase of the α-2,6-ST(*N*) previously described.

Materials and methods

Materials

All reagents were of analytical grade. CMP-[^{14}C]Neu5Ac (262 mCi mmol^{-1} [9.61 GBq mmol^{-1}]) was purchased from Amersham International (UK). Unlabelled CMP-Neu5Ac, 2,3-dehydro-2-deoxy-Neu5Ac, fetuin, Galβ1-3GalNAcα-*p*Np and sialidase from *Clostridium perfringens* were purchased from Sigma Chemical Co. (USA). Ovine submaxillary mucin was obtained from BioCarb (Sweden), Peanut (*Arachis hypogaea*) agglutinin (PNA) and its digoxigenin conjugate, anti-digoxigenin Fab fragments conjugated with alkaline phosphatase, 5-bromo-4-chloro-3-indolyl-phosphate (X-phosphate), 4-nitro blue tetrazolium chloride (NBT) were from Boehringer Mannheim (Germany). Pronase (E3 3.4.21.4 and EC 3.4.24.4) was obtained from Calbiochem (USA) and fluorescein isothiocyanate was from Pierce Chemical Co. (USA). The oligosaccharides Neu5Ac-α2-3Galβ1-3GalNAc-*ol* and Galβ1-3(Neu5Acα2-6)GalNAc-*ol* were the gift of Dr G. Strecker (from our laboratory).

Preparation of acceptors

Glycopeptides from fetuin were obtained after extensive Pronase digestion and *O*-linked glycopeptides were purified by gel permeation on a Bio-Gel P-4 column (Bio-Rad, USA). The different acceptors were desialylated by mild acid hydrolysis with 0.05 M sulfuric acid at 80 °C for 1 h. Sulfuric acid and Neu5Ac were removed either by extensive dialysis against distilled water or by gel permeation on a Bio-Gel P-2 column equilibrated in water. Sugar analysis of desialylated acceptors was carried out by gas/liquid chromatography after methanolysis and trimethylsilylation [29].

Cell culture and preparation of cell homogenates

Transformed derivatives of Fisher rat fibroblasts FR3T3 [30] were previously characterized. Transformation of FR3T3 with *ras* (FR*ras*, FREJ4 in [31]) was performed by transfection with pSV2*neo* EJ plasmid that carries the 6.6 kbp human Ha-*ras*-1 oncogene from the bladder carcinoma cell line EJ [32]. Cells were grown in Dulbecco modified Eagle medium with 10% fetal calf serum and antibiotics, at 37 °C in a humid atmosphere of 5% CO_2 in air. Confluent cultures were harvested by scraping in cold NaCl/P_i (sodium phosphate 10 mM, NaCl 0.15 M, pH 7.4) and washed twice in buffer. Cells were lysed at 0 °C with 1 ml per flask (170 cm^2) of 10 mM sodium cacodylate buffer, pH 6.5, containing 1% Triton X-100, 20% glycerol, 0.5 mM dithiothreitol and 5 mM $MnCl_2$. After 10 min of incubation under continuous stirring, cell homogenates were centrifuged at 10000 × **g** for 15 min, pellets were discarded and the supernatants were used for enzyme assays. The protein concentration was determined using the Lowry [33] modified method [34] with bovine serum albumin as standard. Protein contents of homogenates were 3.5–5 mg ml^{-1}.

Sialyltransferase assays

Incubations were performed for 1–6 h at 37 °C in a final volume of 120 µl under the following conditions: 40 µl cell homogenate, 0.1 M sodium cacodylate, pH 6.5, 1% Triton X-100, 0.1% bovine serum albumin, 0.2 M galactose (as inhibitor of β-galactosidase), 1 mM 2,3-dehydro-2-deoxy-Neu5Ac, 52.9 µM CMP-[^{14}C]Neu5Ac (0.58 GBq mmol^{-1}, 3.67 kBq per 120 µl). Asialofetuin (480 µg corresponding to 1.6 mM of theoretical acceptor sites), asialo-*O*-glycopeptides of fetuin (2.5 mM), or Galβ1-3GalNAcα-*p*NP (4.0 mM) were used as exogenous acceptors. The incorporation into endogenous substrates was negligible under these conditions. The amount of incorporation was assayed as follows: in the case of glycoproteins, the reaction was stopped by addition of 1 ml ice-cold 5% phosphotungstic acid in 2 M HCl. The precipitate was collected on a glass fibre filter and washed extensively with 5% trichloroacetic acid, then with distilled water and ethanol, and processed for scintillation counting. Otherwise, the reaction was stopped by addition of 1 volume of ethanol, samples were centrifuged at 3000 rev min^{-1} for 5 min and supernatants were directly processed for descending paper chromatography using the following solvents:pyridine:ethyl acetate:acetic acid:water (5:5:1:3 by vol) or ethyl acetate:pyridine:water (10:4:3 by vol) in the case of sialyl Galβ1-3GalNAcα-*p*Np products.

Release of [^{14}C]Neu5Ac-labelled glycans from asialofetuin

Asialofetuin (ASFet) (480 μg) was incubated for 4 h under standard conditions of sialyltransferase assay. Aliquots of each incubation mixture were assayed for incorporation of [^{14}C]Neu5Ac as described above. The glycoprotein was separated from CMP-[^{14}C]Neu5Ac and [^{14}C]Neu5Ac by desalting on a Bio-Gel P-2 column (200–400 mesh) equilibrated in 0.1 M pyridine acetate, pH 5.6, and the glycans were released by alkaline treatment under reducing conditions in 0.1 M NaOH containing 1 M $NaBH_4$ for 48 h at 45 °C [35]. At the end of the incubation time, samples were acidified to pH 6 by addition of Dowex 50 × 8 (H^+ form, 20–50 mesh) and evaporated. They were dissolved in methanol and evaporated three times, then taken up in water and applied to a previously calibrated Bio-Gel P-4 column (100 cm × 1.6 cm, 200–400 mesh) equilibrated in 0.1 M pyridine acetate, pH 5.6 [22]. *O*-Glycan fractions were analysed by HPLC on a 5 μm Supercosil LC-NH_2 column run isocratically in 80% acetonitrile: 20% KH_2PO_4 (15 mM), pH 5.2, at 2 ml min^{-1} for 30 min, after which a linear gradient was started to increase the KH_2PO_4 percentage by 0.5% per min up to 30% [36].

Binding of peanut agglutinin to FR3T3 and FRras glycoproteins

Expression of T antigen, Galβ1-3GalNAc(1-*O*)Ser, on FR3T3 and FR*ras* cells was studied by measuring the specific binding of peanut agglutinin [37]. The binding of PNA was also assayed after sialidase treatment in order to estimate the amount of sialylated Galβ1-3GalNAc-*R* sequences by comparison with the binding of PNA on untreated cells. Two different methods were used, either by the binding of fluorescein isothiocyanate labelled PNA (FITC-PNA) on the cell surface of intact cells or by the binding of PNA conjugated with digoxigenin (PNA-dig) after separation of microsomal glycoproteins by SDS-PAGE [38].

Flow cytometry experiments. Confluent FR3T3 and FR*ras* cells were washed and scraped off in NaCl/P_i supplemented with 0.02% NaN_3 (w/v). 10^6 cells ml^{-1} were desialylated with 50 mU ml^{-1} of sialidase from *Clostridium perfringens* in 100 mM phosphate/citrate buffer [39], pH 6.5, 0.1% $CaCl_2$. After 90 min, cells were washed three times and incubated with FITC-PNA (10 μg ml^{-1} in NaCl/P_i). Nonspecific binding was measured by incubating cells with FITC-PNA in the presence of 0.2 M lactose [37]. Data were collected by cytofluorimetry. The light scatter channels were set on linear gains and the fluorescence channels on a logarithmic scale. Five thousand events from each sample were analysed from forward and side angle light scatters for fluorescence intensities with ungated acquisition.

Electrophoresis and glycoprotein staining. SDS-polyacrylamide gel electrophoresis was performed on 5–24% gradient gels under reducing conditions using 30 μg protein from microsomal fractions of FR3T3 and FR*ras* cells per lane. Western blottings were performed according to classical procedures [40], after which proteins were incubated with PNA-dig (10 μg ml^{-1} in NaCl/P_i) for 2 h. Desialylation was performed by treatment of the blots with 50 mU ml^{-1} of sialidase from *Clostridium perfringens* in 50 mM sodium citrate buffer, pH 6.0, 0.9% NaCl, 0.1% $CaCl_2$ for 24 h at 37° C prior to incubations with PNA-dig. Then, the nitrocellulose membranes were incubated for 2 h with anti-digoxigenin Fab fragments conjugated with alkaline phosphatase, and labelled glycoproteins were revealed by NBT/X-phosphate staining. Negative controls were obtained by incubation of PNA-dig together with 0.2 M lactose.

Results

Decrease of CMP-Neu5Ac: Galβ1-3GalNAc-R, α-2,3-sialyltransferase activity in ras*-transformed FR3T3 cells*

In a previous study [21], we have shown a 10-fold increase of the activity of the α-2,6-ST(*N*) in *ras*-transformed cells compared with normal FR3T3 cells. This observation was obtained using different substrates containing only Galβ1-4GlcNAc-*R* acceptor sites (e.g., asialo-α_1-acid glycoprotein). However, this change in the transfer of [^{14}C]Neu5Ac was not observed using ASFet as an acceptor in the sialyltransferase assay. ASFet contains three triantennary complex-type glycans and three *O*-linked oligosaccharides, mainly Galβ1-3GalNAc(α1-*O*)Ser [41] and may be used as an acceptor by four different sialyltransferases acting on *N*-linked or *O*-linked glycans. To elucidate that point, we analysed the sialylation products of ASFet incubated in the standard conditions for sialyltransferase assays with extracts from FR3T3 and FR*ras* cells.

Gel permeation on a Bio-Gel P-4 column [22] resolved the oligosaccharides obtained after β-elimination into two main peaks (Fig. 1) appearing, respectively, at the elution volume of *N*-linked (arrow at position 1) and of *O*-linked glycans of fetuin (arrow at position 2). It appeared that, concomitantly with the increased transfer of [^{14}C]Neu5Ac on *N*-linked chains (peak I), which reflected the elevated activity of the α-2,6-ST(*N*) previously described, the transfer of sialic acid residues on the *O*-linked fraction is greatly decreased when FR*ras* cellular homogenates were used as enzyme source. HPLC analysis of peak II gave only one homogeneous peak which co-migrated with the unlabelled oligosaccharide Neu5Acα2-3Galβ1-3GalNAc-*ol* added as internal marker (data not shown). The α-2,6-ST(*O*)I (EC 2.4.99.3), which is specific for the *R*-GalNAc(α1-*O*)Ser sequence [42], can also accept *O*-linked chains of asialofetuin as an acceptor. The activity of this enzyme, assayed using desialylated ovine submaxillary mucin, was negligible in either normal or *ras*-transformed FR3T3. α-2,6-ST(*O*)II activity was assayed using native fetuin. Most of the *N*-linked chains of native fetuin are trisialylated and *O*-linked chains

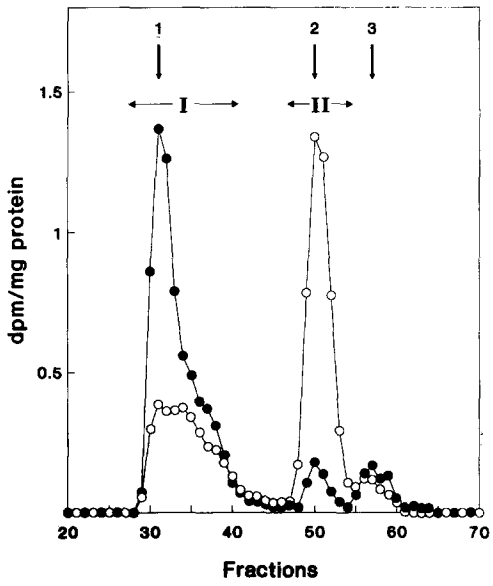

Figure 1. Fractionation by gel permeation of the ^{14}C sialylated glycans obtained from ASFet sialylated by FR3T3 and FR*ras* cellular homogenates. Reduced oligosaccharides were separated on a Bio-Gel P-4 column (100 cm × 1.6 cm) in 0.1 M pyridine acetate, pH 5.6, and eluted with the same buffer at a flow rate of 12 ml h^{-1}. Fractions of 1.85 ml were collected and assayed for radioactivity. The column was previously calibrated with: 1, *N*-glycans of fetuin; 2, mono- and disialylated *O*-glycans of fetuin; 3, Neu5Ac. ○ and ● correspond, respectively, to asialofetuin sialylated by FR3T3 and FR*ras* cellular homogenates.

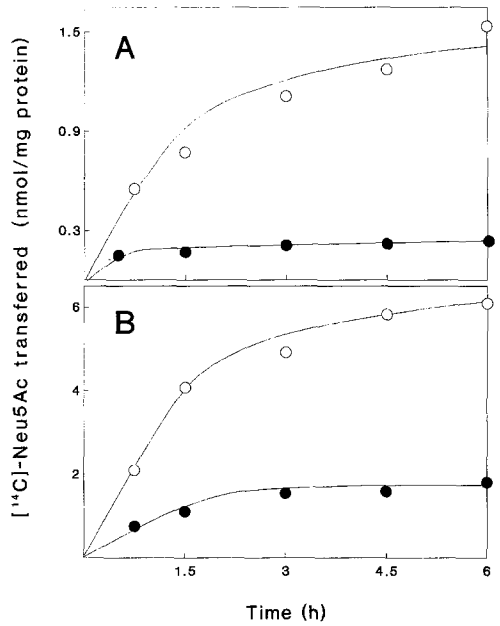

Figure 2. CMP-Neu5Ac:Galβ1-3GalNAc-*R* α-2,3-sialyltransferase activity of FR3T3 and FR*ras* cell homogenates. Incubations were performed in the standard conditions described in the Materials and methods section with A, 2.5 mM asialo-*O*-glycopeptides of fetuin, or B with 4.0 mM Galβ1-3GalNAcα-*p*Np as acceptors. Results are expressed as mean values of two separate experiments in nmol [^{14}C]Neu5Ac residues transferred per mg protein contained in cell homogenate. ○, FR3T3; ●, FR*ras*.

are at least monosialylated. About two thirds of the *O*-linked chains are Neu5Acα2-3Galβ1-3GalNAc(α1-*O*)Ser, while the main remaining structure is the disialyl core 1, Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAc(α1-*O*)Ser. Native fetuin may therefore serve as an acceptor for α-2,6-ST(*O*)II. This activity was present in FR3T3 and FR*ras* cells but no significant difference could be detected between the two cell lines (0.21 nmol mg^{-1} h^{-1} and 0.17 nmol mg^{-1} h^{-1} for FR3T3 and FR*ras*, respectively). This suggested that the decrease of the transfer of [^{14}C]Neu5Ac to the *O*-linked chains of ASFet resulted only from the decrease of the activity of the α-2,3-ST(*O*) and that the pathway used in FR3T3 for the biosynthesis of disialyl core 1 involved the sequenced action of α-2,3-ST(*O*) and of α-2,6-ST(*O*)II. The decrease of the α-2,3-ST(*O*) activity was confirmed by using purified *O*-linked glycopeptides of ASFet and Galβ1-3GalNAcα-*p*Np [43] as acceptors. As indicated in Fig. 2, the incorporation of [^{14}C]Neu5Ac was linear up to 1.5 h and in both cases, the transfer of [^{14}C]Neu5Ac was decreased in FR*ras* homogenates (about sevenfold with purified *O*-linked glycopeptides of ASFet and fourfold with Galβ1-3GalNAcα-*p*Np). These results indicated that the decreased transfer of [^{14}C]Neu5Ac to *O*-linked chains of ASFet was not due to a competitive effect of the different sialyltransferases able to use ASFet as an acceptor and that, concomitantly to the increase of the α-2,6-ST(*N*) activity, the presence of an activated *ras* gene in FR3T3 cells decreased the activity of the α-2,3-ST(*O*).

The decrease of α-2,3-ST(O) activity is supported by a decrease amount of active α-2,3-ST(O) protein in FRras cells

Variable concentrations of Galβ1-3GalNAcα-*p*Np were used to determine kinetic parameters of α-2,3-ST(*O*) in cellular homogenates of FR3T3 and FR*ras*. Dixon plots were used to determine V_{max} and K_M. As indicated in Fig. 3, both cells exhibited the same apparent K_M value (36 ± 0.3 μM) but V_{max} was about fourfold decreased in FR*ras* extracts (0.46 ± 0.10 nmol mg^{-1} h^{-1} in FR*ras* and 1.88 ± 0.17 nmol mg^{-1} h^{-1} in FR3T3). This finding could be explained either by a decrease in the amount of active molecules in *ras*-transformed cells or by the presence of a noncompetitive inhibitor able to decrease the apparent V_{max} in FR*ras* cell extracts. This question may be answered by experiments where Galβ1-3GalNAcα-*p*Np was incubated with CMP-Neu5Ac and with variable mixtures of FR3T3 and FR*ras* cell extracts (from 100% FR3T3 to 100% FR*ras*). The absence of inhibitor would result in a linear decrease in activity corresponding merely to addition of FR3T3 and FR*ras* activities. The presence of inhibitor would lead to a decreasing curve as a consequence of the theoretical equation shown in the legend of Fig. 4. The experimental data (Fig. 4) clearly show that the α-2,3-ST(*O*) activity

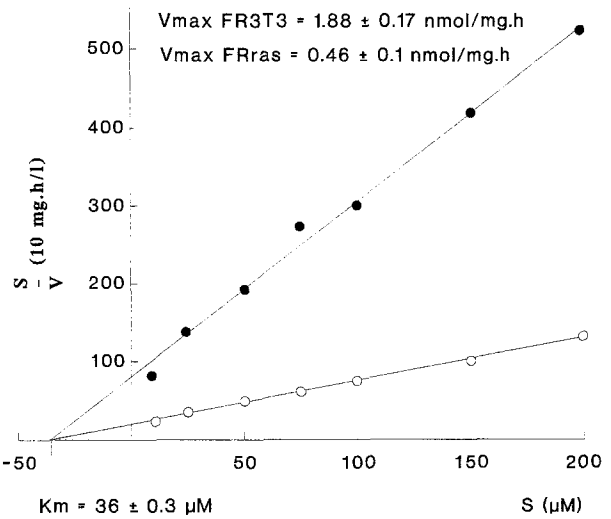

Figure 3. Effect of substrate concentrations on the activity of the CMP-Neu5Ac:Galβ1-3GalNAc-*R* α-2,3-sialyltransferase in FR3T3 and FR*ras* cell homogenates. Complete incubation mixtures as described in the Materials and methods section and containing varying concentrations of Galβ1-3GalNAcα-*p*Np as indicated, were incubated 1.5 h at 37 °C. Kinetic parameters V_{max} and K_M were extracted from Dixon plots (V/[S] versus [S]) by linear regression assisted with the ENZPACK program. The correlation coefficients were 0.998 and 0.982 for FR3T3 and FR*ras*, respectively. ○, FR3T3; ●, FR*ras*.

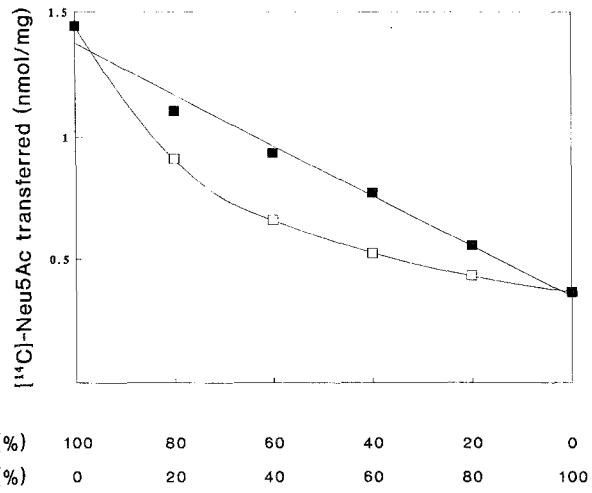

Figure 4. Effect of FR3T3 and FR*ras* cell homogenates ratio on the activity of the CMP-Neu5Ac:Galβ1-3GalNAc-*R* α-2,3-sialyltransferase. Incubation mixtures containing 4 mM Galβ1-3GalNAcα-*p*Np and FR3T3 and FR*ras* cell homogenates in ratios varying as indicated were incubated 1.5 h at 37 °C. Results are expressed in nmol [^{14}C]Neu5Ac transferred per mg protein in cell homogenates and per hour. Linear regression (■) calculated from experimental data gave a correlation coefficient equal to 0.967. The theoretical curve (□) was calculated according to the percentage p of FR*ras* extract present in the incubation mixture from the equation:

$$V(p) = \frac{V_{max}[S]}{K_M + [S]} \times \frac{1}{1 + \left[\left(\frac{V(0)}{V(100)} - 1\right) \times \frac{p}{100}\right]}$$

in which V_{max} and K_M were the kinetic parameters of α-2,3-ST(O) in FR3T3 cell extracts (Fig. 3), $V(0)$ was the activity measured with 100% FR3T3 and $V(100)$ was the activity measured with 100% FR*ras*.

decreased linearly from 100% FR3T3 (1.52 ± 0.13 nmol mg^{-1} h^{-1} to 100% FR*ras* (0.41 ± 0.07 nmol mg^{-1} h^{-1}) and the comparison of the linear regression plot, calculated from experimental values, to the theoretical inhibition curve made clear the fact that the observed decrease of α-2,3-ST(O) activity in *ras*-transformed cells was due to the decrease of the amount of α-2,3-ST(O) protein and not to the presence of an inhibitor.

No correlation between the decreased α-2,3-ST(O) activity and the binding of PNA at the cell surface of ras-transformants

In an attempt to correlate the decrease of the α-2,3-ST(O) activity with changes in core 1 sialylation, cells were incubated with FITC-PNA. Peanut agglutinin binds with a high specificity to the T antigen, Galβ1-3GalNAc(1-*O*)Ser [37]. As indicated in Fig. 5, the binding of FITC-PNA on the membrane of both cells was weak without previous desialylation, and no significant difference could be detected between FR3T3 and its *ras*-transformed derivative. Desialylation of the cell surface with the sialidase from *Clostridium perfringens* induced a shift of the fluorescence peak to higher level of fluorescence, indicating that a large proportion of the T antigen was sialylated in FR3T3 cells. Moreover, the fluorescence intensity was in the same range for FR3T3 and FR*ras* cells, which suggested that no large difference in the sialylation of core 1 appeared in *ras*-transformed cells, while the activity of the α-2,3-ST(O) was fourfold decreased. This observation was confirmed by the determination of the binding of PNA-dig on the glycoproteins obtained from microsomal fractions of FR3T3 and FR*ras* and separated by SDS-PAGE (Fig. 6). Western blots were incubated with the lectin without (lanes 1a to 3a) or with previous treatment of the blots with 50 mU per ml sialidase from *Clostridium perfringens* (lanes 1b to 3b) in order to visualize sialyl-T antigens (lane 4 corresponds to 5 µg fetuin used as a control for the desialylation). The specificity of the binding of PNA-dig was checked by the incubation of the lectin in the presence of 0.2 M lactose (lanes 1c to 3c). Before or after desialylation, the staining of the labelled glycoproteins was similar for both cell lines and significant differences could not be found in the amount of core 1 or in the sialylation of these structures.

Discussion

We have demonstrated that the transformation of FR3T3 cells by the human Ha-*ras* oncogene induces a decrease of the activity of the CMP-Neu5Ac:Galβ1,3GalNAc-*R* α-2,3-sialyltransferase. This observation has been demonstrated

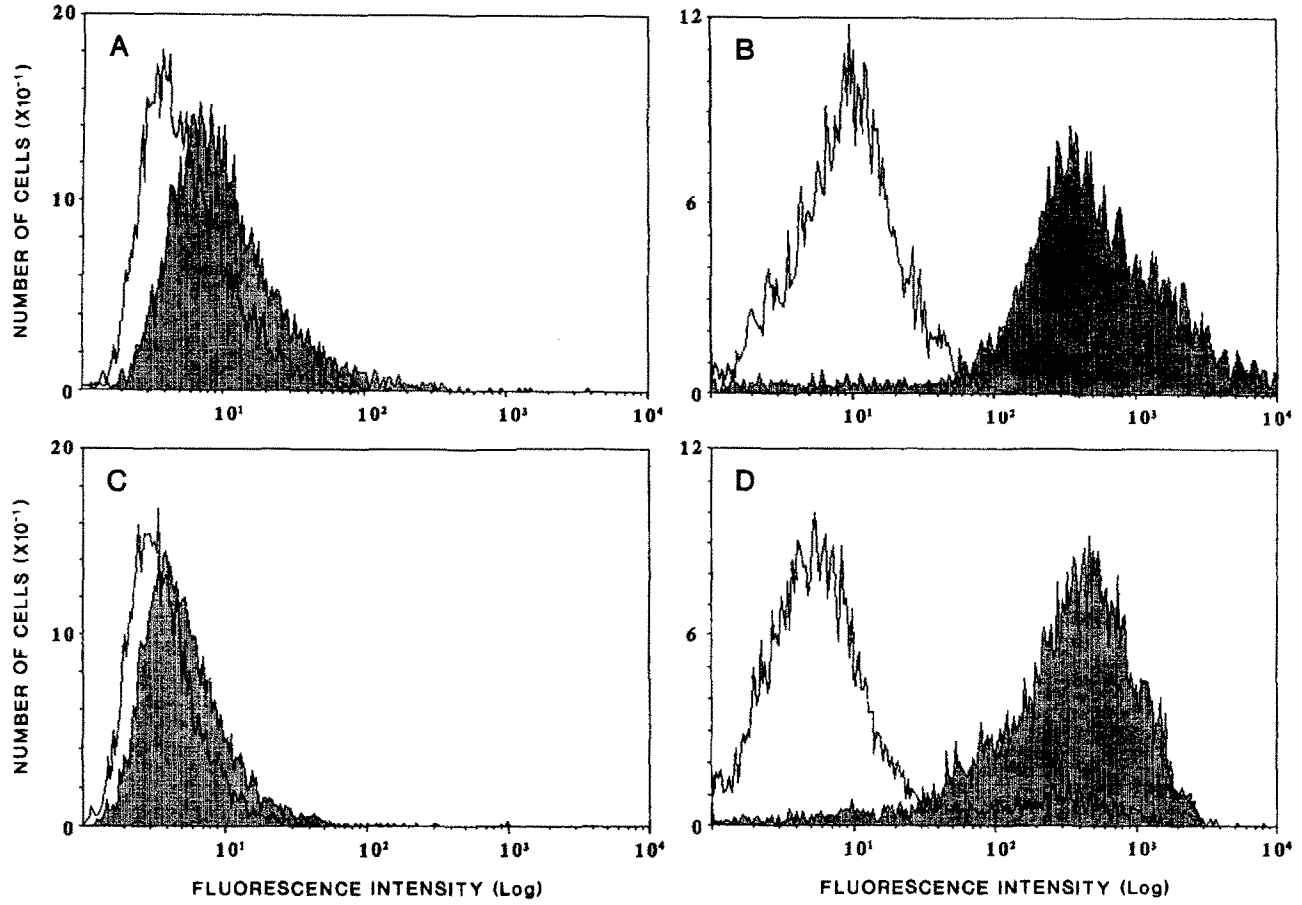

Figure 5. Binding of FITC-PNA at the cell surface of FR3T3 and FR*ras* cells analysed by FACS. FR3T3 cells (*A*, *B*) and FR*ras* cells (*C*, *D*) were incubated with 10 μg ml^{-1} FITC-PNA (hatched area) without (*A*, *C*) and with (*B*, *D*) previous desialylation with *Clostridium perfringens* sialidase. For each assay, the inhibition of the binding of the lectin with 0.2 M lactose is presented (open area).

clearly using different specific acceptors of this *O*-glycan core 1 sialyltransferase. α-2,6-ST(*O*)I was not expressed in these cells and the activity for the α-2,6-ST(*O*)II, which has a strict acceptor specificity of the monosialyl sequence Neu5Acα2-3Galβ1-3GalNAc-*R*, was not changed in *ras*-transformed cells. These results suggest that a classical pathway, previously described in liver [44], is used in FR3T3 cells for the biosynthesis of disialyl core 1, involving the ordered action of the α-2,3-ST(*O*) and α-2,6-ST(*O*)II. In a previous study with the same cellular model [21], we have demonstrated that the activity of the α-2,6-ST(*N*) was about 8–10-fold increased in *ras*-transformed cells, while the activity of the α-2,3-ST(*N*/*O*) remained unchanged. From these different data, it appears that the presence of the *ras* gene modifies the activity of two distinct sialyltransferases in an opposite manner.

According to the kinetic parameters of α,2,3-ST(*O*) in FR3T3 and FR*ras* cells and to the results of mixing experiments, the diminished activity seems to reflect a decrease in the number of active α-2,3-ST(*O*) molecules rather than the presence of an inhibitor in FR*ras* cells. The elevation of the α-2,6-ST(*N*) activity previously described [21] was demonstrated using rat liver α-2,6-ST(*N*) cDNA as a probe to be the consequence of an increase in the transcription rate of the related gene. Consequently, it will be of a great interest to determine if the decrease of α-2,3-ST(*O*) comes from a decrease in the amount of specific transcripts in *ras*-transformants.

Several other studies have indicated changes in glycosyltransferase activities in *ras*-transformed cells. For example, the increased activity of *N*-acetylglucosaminyltransferase V was demonstrated in *ras*-transformed rat2 cell lines [45]. UDP-G1cNAc:Galβ1-3GalNAc-*R* (GlcNAc to GalNAc) β1,6-GlcNAc-transferase, which controls *O*-linked polylactosaminoglycan biosynthesis, is also increased in *ras*-transformed rat2 and metastatic murine cell lines [12]. Finally, Easton *et al.* [23] have recently defined the changes in glycosyltransferase activity occurring in NIH3T3 overexpressing *N-ras*, and they have underlined the high increase of the activity of the UDP-GlcNAc:Galβ1-4GlcNAc-*R* (GlcNAc to Gal), β-1,3-*N*-acetylglucosaminyltransferase, leading to a higher proportion of polylactosaminoglycan chains.

How does the *ras* oncogene regulate the expression of these different glycosyltransferases, such as α-2,6-ST(*N*) and

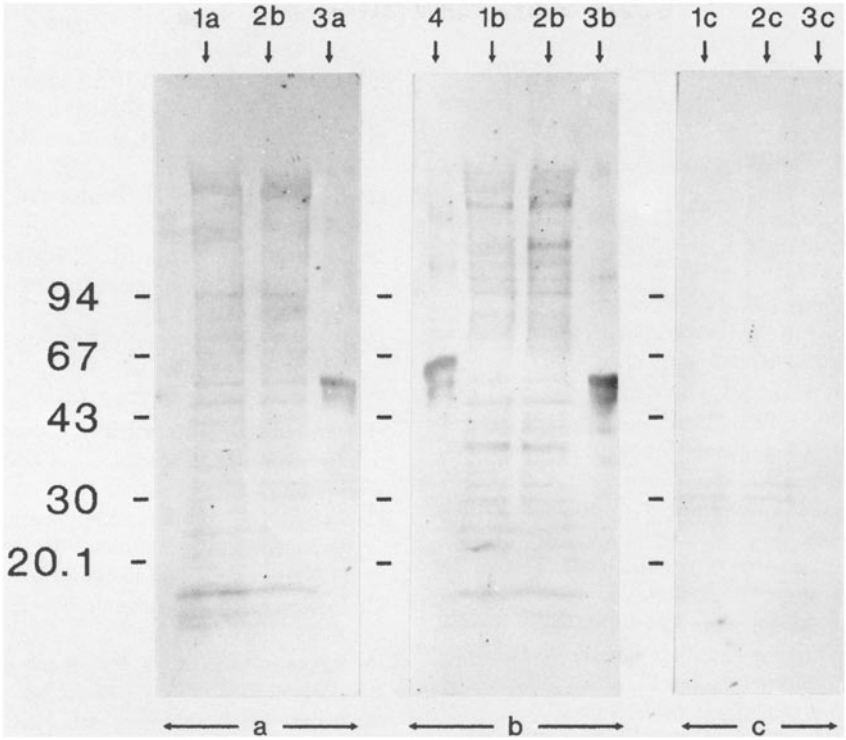

Figure 6. Binding of PNA-dig on glycoproteins from FR3T3 and FR*ras* cells separated by SDS-PAGE. 30 μg protein from microsomal fractions of FR3T3 and FR*ras* were separated by SDS-PAGE and stained with PNA-dig as described in the Materials and methods section. Lanes 1, FR3T3 cellular homogenate; lanes 2, FR*ras* cellular homogenate; lanes 3, ASFet (5 μg). Blots were incubated with the lectin without (*a*) and with (*b*) previous desialylation. Lane 4 corresponds to 5 μg fetuin used as a control of desialylation. (*c*) The right part of the blot was treated with sialidase and incubated with 0.2 M lactose for the negative control of the binding of the lectin. The left lane indicates the positions of the molecular weights of marker proteins: phosphorylase b (94 000), albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100).

α-2,3-ST(*O*) in FR3T3? Some reports have shown that Ha-*ras* is able to modulate gene expression indirectly, either positively or negatively, at the transcriptional level [46] and, in particular, this has been demonstrated for α-2,6-ST(*N*) [21]. However, the mechanism of action of $p21^{ras}$ is unclear, and even if cooperation between *ras* oncogenes and nuclear oncogenes is now described, many steps remain to be understood in the cascade of events leading to changes in the transcription rate of *ras*-regulated genes.

Previous studies have indicated the increased activity of α-2,3-ST(*O*) in chronic myelogenous leukemia granulocytes [24, 25] and transformation of NIH3T3 cells with the Ha-*ras* oncogene increased the activity of α-2,6-ST(*N*) but did not modify the activity of α-2,3-ST(*O*) [22]. These different observations seem to indicate that changes in α-2,3-ST(*O*) activity in transformed cells depend on the cellular model and, in particular, would be species and/or tissues specific.

While α-2,3-ST(*O*) activity was at least fourfold decreased in FR*ras* cells, no change in the binding of PNA was observed on the cell surface of *ras*-transformed FR3T3 cells, suggesting that no change in the sialylation of the *O*-glycan core 1 appeared in FR*ras* cells. The significance of this observation remains unclear but it seems to indicate that while the amount of α-2,3-ST(*O*) appears to be fourfold decrease in *in vitro* experiments, the remaining activity can be sufficient to sialylate in the same proportion the endogenous acceptors. In that way, α-2,3-ST(*O*) expression would not be a limiting factor for the biosynthesis of sialyl core 1.

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