Inhibition of myoblast fusion by the glucosidase inhibitor *N*-methyl-1-deoxynojirimycin, but not by the mannosidase inhibitor 1-deoxymannojirimycin.

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The effects of *N*-linked-oligosaccharide-processing inhibitors on the fusion of rat L₆ myoblasts to form myotubes were examined. The glucosidase inhibitor *N*-methyl-1-deoxynojirimycin (MDJN) greatly inhibited fusion, whereas the mannosidase inhibitor 1-deoxymannojirimycin (ManDJN) had relatively little effect, although both compounds prevented the formation of *N*-linked complex oligosaccharides. These results indicate that complex oligosaccharides on glycoproteins do not play a role in myoblast fusion. With MDJN, high-mannose oligosaccharides containing three glucose residues and seven to eight mannose residues were found at the cell surface, whereas with ManDJN, non-glucosylated high-mannose oligosaccharides with seven to nine mannose residues were obtained. These results indicate that the persistence of glucose residues on high-mannose oligosaccharides may be responsible for the inhibition of fusion. It is suggested that glucose either masks the cell-surface recognition process leading to fusion or prevents the cell-surface expression of specific glycoprotein(s) essential to the fusion process.

INTRODUCTION

During the terminal differentiation of skeletal muscle, mononucleate myoblasts fuse to form myotubes. These elongate, multinucleate, cells subsequently differentiate further to form striated muscle fibres. The precise sequence of events controlling the process of myoblast fusion, and the molecular components of the membrane involved in the fusion process, are not yet understood. Studies on the formation of myoblast aggregates in suspension suggest that fusion is a multi-step process, with discrete stages of myoblast recognition, adhesion and membrane union, each involving different or additional molecules (Knudsen & Horwitz, 1977). Several studies have shown that there are alterations in cell-surface glycoproteins (Marino *et al.*, 1980; Wahrmann *et al.*, 1980; Walsh & Phillips, 1981; Boitani *et al.*, 1982; Cates *et al.*, 1984a,b; Holland *et al.*, 1984) coincident with myoblast fusion, and it has been suggested that glycoproteins are involved in the adhesion stage preceding fusion (Knudsen, 1985). There is no direct evidence that any specific glycoproteins play an essential role in the fusion process. There are indications, however, that glycoproteins containing asparagine-linked (*N*-linked) oligosaccharides may be causally involved in myoblast fusion. Fusion is blocked in concanavalin A-resistant mutants of L₆ myoblasts (Parfett *et al.*, 1983; Cates *et al.*, 1984a). Furthermore, tunicamycin, which interferes with the synthesis of dolichyl pyrophosphate oligosaccharides required for the formation of all *N*-linked oligosaccharides, greatly inhibits myoblast fusion (Gilfix & Sanwal, 1980; Olden *et al.*, 1981).

N-Linked oligosaccharides are present on glycoproteins in two major forms, namely complex and high mannose, which both arise from processing of the same precursor oligosaccharide, Glc₃Man₉GlcNAc₂. The initial steps in this processing are the removal of the glucose residues by two specific rough-endoplasmic-reticulum α -glucosidases, followed by removal of a variable number of mannose residues by rough-endoplasmic-reticulum and Golgi α -mannosidases. In the case of complex oligosaccharides, terminal glycosylation by specific glycosyltransferases subsequently occurs, in the Golgi. [for a review, see Kornfeld & Kornfeld (1985)]. Since tunicamycin inhibits the synthesis of all *N*-linked oligosaccharides, its use does not give any information regarding the possible preferential involvement of the different types of *N*-linked oligosaccharides in the fusion process. Furthermore, the complete inhibition obtained with tunicamycin may result in increased proteolytic degradation of the non-glycosylated proteins (Olden *et al.*, 1981). Recent studies indicate that compounds which interfere with different stages in oligosaccharide processing can be used to modify the structure of *N*-linked oligosaccharides on glycoproteins [for a review, see Schwarz & Datema (1984)]. We have shown that the glucose analogue DJN is a potent inhibitor of processing α -glucosidases I and II and that it is active in intact cells (Saunier *et al.*, 1982). It has since been found that its derivative, MDJN, is a more useful inhibitor of the glucosidases (Romero *et al.*, 1983, 1985a,b; Hettkamp *et al.*, 1984) and that the corresponding mannose analogue, ManDJN, inhibits processing Golgi α -mannosidase I (Bischoff & Kornfeld, 1984; Fuhrmann *et al.*, 1984; Romero *et al.*, 1985c).

Abbreviations used: DJN, 1-deoxynojirimycin; MDJN, *N*-methyl-1-deoxynojirimycin; ManDJN, 1-deoxymannojirimycin; endo-H, endo- β -*N*-acetylglucosaminidase H; DMEM, Dulbecco's modified Eagle medium; *R*, the retention time of each oligosaccharide divided by the retention time of standard Glc₃Man₉GlcNAc; BSA, bovine serum albumin.

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Since MDJN and ManDJN prevent the formation of complex *N*-linked oligosaccharides, they can be used to investigate the relative function of high-mannose and complex oligosaccharides in biological processes. In the present study we examined the effects of these processing inhibitors on the fusion of L_6 rat myoblasts and on the synthesis of *N*-linked oligosaccharides in these cells. It was found that MDJN prevents myoblast fusion, whereas ManDJN has relatively little effect on fusion.

MATERIALS AND METHODS

Materials

L_6 myoblasts were obtained through the courtesy of Dr. B. D. Sanwal, University of Western Ontario, London, Ontario, Canada; these were a subclone, L_6-9 (Ball *et al.*, 1979) of the line originally isolated by Yaffe (1968). DMEM, fetal-calf serum and horse serum were obtained from Gibco, Grand Island, NY, U.S.A.; D-[2- 3 H]mannose (specific radioactivity, 27 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.); Pronase CB was from Calbiochem, La Jolla, CA, U.S.A.; endo-H was from Miles Laboratories, Elkhart, IN, U.S.A.; jack-bean α -mannosidase was from Sigma, St. Louis, MO, U.S.A.; MDJN was obtained from Dr. Y. Aoyagi, Nippon Sinyaku Co., Kyoto, Japan, and ManDJN from Dr. G. Kinast, Bayer AG, Wuppertal, Germany. The oligosaccharide standards were prepared as described previously (Kilker *et al.*, 1981).

Estimation of fusion of L_6 myoblast cells

The cells were seeded at a concentration of 10^4 cells/ml in triplicate in 24-well dishes in 0.5 ml of DMEM containing 10% (v/v) horse serum and either MDJN or ManDJN, as indicated. After 7 days in culture the cells were fixed with 2.5% glutaraldehyde and were stained with haematoxylin and eosin. The nuclei were counted in ten random fields at 400 $\times$ magnification and the percentage of the total nuclei in myotubes was determined. Only cells containing at least three nuclei were counted as myotubes.

Labelling of cells

L_6 rat myoblast cells, plated in duplicate 100 mm-diameter dishes at a density of 3×10^5 cells, were cultured in 10 ml of DMEM containing 1 g of glucose/l, 5% (v/v) fetal-calf serum and 5% horse serum. When present, the inhibitors MDJN and ManDJN were added at the time of seeding to yield a final concentration of 2 mM. After cells had spent 3 days in culture, 10 ml of fresh medium containing D-[2- 3 H]mannose at a concentration of 15 μ Ci/ml were added with or without inhibitor. After 24 h of labelling the cells were washed three times with 10 ml of Dulbecco's phosphate-buffered saline, pH 7.4. The cells were then exposed to 3 ml of 0.1% trypsin/0.05% EDTA in phosphate-buffered saline for 10 min at room temperature. After trypsin treatment the suspension was centrifuged at 800 *g* for 5 min to remove any detached cells and the supernatant was freeze-dried. This material, which is enriched in cell-surface components, is subsequently referred to as the 'cell-surface-glycopeptide fraction'.

Fractionation of glycopeptides and oligosaccharides

The cell-surface glycopeptides were exhaustively digested for 24 h at 60 $^{\circ}$ C in 1 ml of Tris/HCl buffer, pH 8.0, containing 1 mM-CaCl₂, 2 mg of Pronase/ml and

25 μ g each of cycloheximide and chloramphenicol/ml. The glycopeptides were chromatographed on a column (1 cm $\times$ 100 cm) of Bio-Gel P-6 in 0.1 M-pyridine/acetate/0.5 mM-Na₂N₃, pH 5.0, with BSA (to determine the void volume) and mannose. Fractions (1 ml) were collected and assayed for radioactivity. The phenol/H₂SO₄ reaction was used to locate mannose, and A_{280} to locate BSA. The glycopeptide fractions were pooled, freeze-dried and treated for 24 h with 2 munits of endo-H in 200 μ l of 0.1 M-sodium citrate, pH 5.5, containing 25 μ g each of cycloheximide and chloramphenicol/ml. The products obtained were chromatographed on the same column of Bio-Gel P-6 as that described above. The endo-H-sensitive oligosaccharides were pooled, concentrated, and chromatographed with 14 C-labelled oligosaccharide standards on a column (25 cm $\times$ 0.46 cm) of 5 μ m-particle-size Aminospherisorb (Phase Separations; packed by Chromatography Sciences Co., Ville Mont Royal, Quebec, Canada) with a Varian model 5000 liquid chromatograph (Romero *et al.*, 1985a). Filtered samples (100 μ l) dissolved in acetonitrile/water (1:1, v/v) were applied to the column previously equilibrated with the starting solvent. Elution was performed isocratically at 1 ml/min for 20 min with acetonitrile/water (3:2, v/v), followed by a linear gradient to acetonitrile/water (21:29, v/v) for 95 min. The water contained 15 mM-potassium phosphate and 5 mM-Na₂N₃ adjusted to pH 5.0. Fractions (1 ml) were collected and assayed for radioactivity with 4 ml of Scintiverse (Fisher Scientific) as scintillation fluid and an LKB model 1218 Rackbeta liquid-scintillation counter equipped with a d.p.m. calculation program (Romero *et al.*, 1985a).

α -Mannosidase treatment

The endo-H-released oligosaccharides obtained from the Bio-Gel P-6 column were freeze-dried and then incubated for 72 h at 37 $^{\circ}$ C in 200 μ l of 50 mM-sodium acetate buffer, pH 5.0, containing 100 mM-NaCl, 0.1 mM-ZnSO₄ and 0.02% Na₂N₃. Jack-bean α -mannosidase (2 units) was added at 0, 24 h and 48 h of incubation. After 72 h of incubation the samples were boiled for 3 min, centrifuged, and the supernatants were desalted through coupled columns of AG 50 W (X8, H⁺ form, 200–400 mesh) and AG1 (X8, formate form, 200–400 mesh) resins. The α -mannosidase treatment was repeated and the samples were subjected to h.p.l.c. as described above (Romero *et al.*, 1985a).

RESULTS

Effects of MDJN and ManDJN on myoblast fusion

When rat L_6 myoblast cells were subcultured, they first went through a period of rapid growth until they reached confluence after about 4 days in culture. Fusion of myoblasts to form myotubes usually began on day 4 of culture, and reached a maximum after about 6–7 days. On day 7, about 75% of the nuclei were found in myotubes. Addition of MDJN at the time of seeding greatly inhibited fusion, so that only 23% of the nuclei were found in myotubes after 7 days in culture. In contrast, the addition of ManDJN under the same conditions only had a small inhibitory effect on fusion, and about 60% of the nuclei were recovered in myotubes (Figs. 1 and 2). Addition of MDJN 1 and 2 days after seeding still greatly inhibited fusion, but this inhibition became less and less pronounced with delayed addition of

Fig. 1. Effects of MDJN and ManDJN on myoblast fusion

The L_6 myoblast cells were grown in triplicate with and without either MDJN (▨) or ManDJN (■), which were added at a final concentration of 2 mM at the time (days after plating) indicated. After 7 days in culture, the cells were fixed with 2.5% (w/v) glutaraldehyde and were stained with haematoxylin and eosin. The percentage of the total nuclei in myotubes was determined by counting nuclei at $400\times$ magnification. Only cells containing more than two nuclei were counted as myotubes. Values are means, and the bars indicate the S.D. for three independent experiments. The corresponding value for control cells grown in the absence of inhibitor was $74 \pm 4\%$ nuclei in myotubes.

this compound. Addition of MDJN 4 days after seeding had little effect on the ability of the cells to form myotubes. The inhibitory effect of MDJN was concentration-dependent, with a maximum inhibition observed at a concentration of 0.5 mM (Fig. 3). Castanospermine, another glucosidase inhibitor (Pan *et al.*, 1983), also inhibited fusion; at concentrations of 10, 50 and 100 $\mu\text{g}/\text{ml}$, about 60, 47 and 43% of the nuclei respectively were associated with myotubes after 7 days in culture. The simultaneous addition of leupeptin (25–100 $\mu\text{g}/\text{ml}$), which had been shown previously to reverse the effect of tunicamycin (Olden *et al.*, 1981), did not reverse the inhibition observed with MDJN. The addition of 0.5 mM-MDJN at the time of seeding inhibited growth of the cells only about 10–15%, whereas the addition of ManDJN did not have any significant effect.

Effect of MDJN and ManDJN on cell-surface glycopeptides

The effects of MDJN and ManDJN on the structure of *N*-linked oligosaccharides present on cell-surface glycoproteins was examined after 3 days of exposure to these compounds. Cell-surface material was released with mild trypsin treatment after 24 h incubation with labelled mannose, and labelled glycopeptides, obtained by exhaustive Pronase digestion, were subjected to gel filtration before and after treatment with endo-H to distinguish glycopeptides containing complex oligosaccharides from high-mannose oligosaccharides. In control cells, two glycopeptide fractions were obtained (Fig. 4a) ($K_{\text{av.}} = 0.25$ and 0.42). Treatment with endo-H did not affect the elution of the higher- M_r glycopeptides, and caused a shift in the elution of the smaller glycopeptides from $K_{\text{av.}} = 0.42$ to 0.67, thereby demonstrating that this

fraction contains high-mannose oligosaccharides (Fig. 4d). In the MDJN-treated cells, the cell-surface glycopeptides consisted of a major fraction ($K_{\text{av.}} = 0.35$) with a poorly resolved shoulder eluted earlier (Fig. 4b). After endo-H treatment (Fig. 4e), the elution of the major labelled fraction was shifted to lower- M_r material ($K_{\text{av.}} = 0.35$ to 0.53). These results indicate that MDJN greatly inhibited the synthesis of *N*-linked complex oligosaccharides. In ManDJN-treated cells, the major glycopeptide fraction ($K_{\text{av.}} = 0.44$) was completely converted by endo-H to lower- M_r material ($K_{\text{av.}} = 0.61$) (cf. Figs. 4c and 4f). These results demonstrate that ManDJN completely inhibited the synthesis of complex oligosaccharides.

The labelled oligosaccharides released by endo-H and obtained from the Bio-Gel P-6 column were further fractionated by h.p.l.c. before and after exhaustive treatment with jack-bean α -mannosidase to distinguish between glucosylated and non-glucosylated high-

Fig. 2. Effects of MDJN and ManDJN on the appearance of L_6 myoblast cells

The cells were cultured for 7 days after seeding and were fixed with 2.5% glutaraldehyde and then stained with haematoxylin and eosin. A, Control cells; B, MDJN-treated cells; C, ManDJN-treated cells. When present, MDJN and ManDJN were added at the time of seeding to a final concentration of 2 mM. Magnification $\times 160$. The bar represents 100 μm .

Fig. 3. Effect of MDJN concentration on myoblast fusion

Rat L_6 myoblast cells were seeded in triplicate in the presence of different concentrations of MDJN, as indicated. After 7 days in culture, the cells were fixed and stained. The percentage of the total nuclei present in myotubes was determined as described in the Materials and methods section. Values shown are means, and the bars indicate the S.D. for three independent experiments.

mannose oligosaccharides as described previously (Romero *et al.*, 1985a). The endo-H-sensitive oligosaccharides obtained from control cells were resolved into five oligosaccharides, which were eluted earlier than the glucosylated standards and corresponded, in their elution position, to $\text{Man}_{5-9}\text{GlcNAc}$ oligosaccharides (Fig. 5a). None of these oligosaccharide fractions contained glucose residues, since all the radioactivity was eluted with mannose after jack-bean α -mannosidase treatment

(Fig. 5d). The endo-H-sensitive oligosaccharides obtained from MDJN-treated cells consisted of two major fractions with $R = 0.89$ and 0.79 (Fig. 5b). After treatment of the mixture with jack-bean α -mannosidase, only 45% of the radioactivity was eluted with mannose, and about 45% of the radioactivity was recovered in an oligosaccharide with $R = 0.42$ (Fig. 5e). This elution position corresponded to the elution of the oligosaccharide obtained by a similar treatment of standard glucose-labelled $\text{Glc}_3\text{Man}_7\text{GlcNAc}$ with jack-bean α -mannosidase. These results demonstrate that the two major oligosaccharides formed in the presence of MDJN contain three glucose residues and, from their relative elution position (Romero *et al.*, 1985a), consist of $\text{Glc}_3\text{Man}_7\text{GlcNAc}$ and $\text{Glc}_3\text{Man}_8\text{GlcNAc}$; there was also a trace of $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ present (Fig. 5b). In ManDJN-treated cells the major oligosaccharides correspond, in their elution position, to $\text{Man}_{7-9}\text{GlcNAc}$ (Fig. 5c); these are not glucosylated, since all the radioactivity was eluted with mannose after treatment with jack-bean α -mannosidase (Fig. 5f).

To ensure that the inhibition of fusion by MDJN was not due to an effect of the inhibitor on the synthesis of the dolichyl pyrophosphate oligosaccharide precursor, the lipid-linked oligosaccharides formed during a 2 h pulse-labelling with $[2\text{-}^3\text{H}]\text{mannose}$ were examined by h.p.l.c. as described by Romero *et al.* (1985b) after 3 days in culture. The synthesis of $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$, the major oligosaccharide product, was not affected by MDJN (results not shown).

DISCUSSION

The present results demonstrate that fusion of L_6 rat myoblasts in culture is greatly inhibited by MDJN, but is not significantly affected by ManDJN. Since both

Fig. 4. Effects of inhibitors on cell-surface glycopeptides

Bio-Gel P-6 chromatography of glycopeptides released by trypsin treatment of L_6 cells after a 24 h incubation with D- $[2\text{-}^3\text{H}]\text{mannose}$. The cells were allowed to grow for 3 days in the presence or absence of inhibitor before the addition of labelled mannose. (a, d) Control cells; (b, e) MDJN-treated cells; (c, f) ManDJN-treated cells. The samples were chromatographed before (a, b, c) and after (d, e, f) endo-H treatment. K_{av} values are given above the peaks.

Fig. 5. H.p.l.c. of endo H-sensitive oligosaccharides

Labelled oligosaccharides ($K_{av.} = 0.5-0.6$) released by endo-H treatment of cell-surface glycopeptides after 24 h labelling with D-[2- 3 H]mannose were obtained from a Bio-Gel P-6 column as described for Fig. 4, and were fractionated by h.p.l.c. as described in the Materials and methods section. (a, d) Control cells; (b, e) MDJN-treated cells; (c, f) ManDJN-treated cells. The samples were chromatographed before (a, b, c) and after (d, e, f) exhaustive treatment with jack-bean α -mannosidase. The arrows indicate the positions of the 14 C-labelled standard oligosaccharides: 1, $\text{Glc}_1\text{Man}_6\text{GlcNAc}$; 2, $\text{Glc}_2\text{Man}_6\text{GlcNAc}$; 3, $\text{Glc}_3\text{Man}_6\text{GlcNAc}$. The R values are indicated above each peak. Mannose and disaccharide ($\text{Man}\beta 1-4\text{GlcNAc}$) peak are eluted together in fraction 6 or 7.

compounds were shown to prevent the synthesis of N -linked complex oligosaccharides in these cells, the results indicate that complex oligosaccharides do not play a role in myoblast fusion. In fact, as observed previously in other cells (Romero *et al.*, 1985a,c), the inhibition of complex-oligosaccharide synthesis obtained with ManDJN is more pronounced than that observed with MDJN, and yet ManDJN has relatively little effect on fusion. This conclusion regarding the lack of involvement of complex oligosaccharides is in agreement with previous studies on lectin-resistant myoblast mutants in which it was found that wheat-germ-resistant mutants lacking sialic acid and galactose residues on their glycoproteins remain fusion-competent (Parfett *et al.*, 1983; Gilfix & Sanwal, 1984).

Although MDJN and ManDJN both prevent complex-

oligosaccharide synthesis, the difference in their effects on myoblast fusion may be due to the fact that the glycoproteins formed in the presence of each compound contain different high-mannose oligosaccharides. With MDJN, high-mannose oligosaccharides containing three glucose residues, primarily $\text{Glc}_3\text{Man}_7\text{GlcNAc}$ and $\text{Glc}_3\text{Man}_8\text{GlcNAc}$, are found at the cell surface. Such glucosylated oligosaccharides are not present on the cell surface of untreated cells. With ManDJN, on the other hand, high-mannose oligosaccharides similar to those of untreated cells are present at the cell surface, the only difference being an increased proportion of the oligosaccharides containing eight and nine mannose residues compared with untreated cells. These results are consistent with the mode of action previously described for these two inhibitors; MDJN affects the early steps of

processing in the rough endoplasmic reticulum by preventing the action of α -glucosidases, whereas ManDJN acts at a later stage by inhibiting Golgi α -mannosidase I.

The present observations indicate that the persistence of glucose residues on high-mannose oligosaccharides may be responsible for the inhibition of fusion observed with MDJN. The fact that castanospermine, another processing inhibitor with a mode of action similar to that of MDJN (Pan *et al.*, 1983), also inhibits fusion further supports this concept. Two possible explanations for this effect of glucose can be envisaged. It may be that high-mannose oligosaccharides on one or more glycoproteins at the cell surface are intimately involved in the recognition process leading to adhesion and fusion of the myoblasts; in this case the presence of glucose residues on these oligosaccharides would directly mask this recognition. The other possibility is that the protein domain of specific cell-surface glycoprotein(s) is essential for recognition, and that the persistence of glucose residues on the specific glycoprotein(s) may prevent their appearance at the cell surface. Olden *et al.* (1981) previously reported that inhibition of fusion with tunicamycin was reversed by the proteinase inhibitor leupeptin; although this observation was not confirmed (Cates *et al.*, 1984b), this reversal indicated that the carbohydrate groups are not directly involved in the process of fusion. Since, in the present work, leupeptin had no effect on the inhibition of fusion observed in the presence of MDJN, it is unlikely that the effect of MDJN results from increased proteolytic degradation of glycoproteins essential in the fusion process. It has been shown previously that DJN treatment can interfere with the translocation from rough endoplasmic reticulum to Golgi and with the secretion of some glycoproteins, and not of others (Gross *et al.*, 1983; Peyrieras *et al.*, 1983; Lodish & Kong, 1984). More recently, it was found that both MDJN and castanospermine prevent the cell-surface expression of the *v-fms*-oncogene-encoded glycoprotein, thereby reversing the 'in vitro' tumour phenotype (Nichols *et al.*, 1985). These results indicate that preventing glucose removal from certain glycoproteins interferes with their subsequent translocation to the Golgi and therefore to the cell surface. The possibility must be considered, therefore, that the glycoprotein(s) involved in fusion are not properly targetted to the cell surface in the presence of MDJN but are normally inserted into the membrane in the presence of ManDJN.

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REFERENCES

- Ball, E. H., Narindrasorasak, S. & Sanwal, B. D. (1979) *Can. J. Biochem.* **57**, 1220–1228
- Bischoff, J. & Kornfeld, R. (1984) *Biochem. Biophys. Res. Commun.* **125**, 324–331
- Boitani, C., Cossu, G., Bouche, M., Molinaro, M. & Pacifici, M. (1982) *Exp. Cell Res.* **138**, 489–494
- Cates, G. A., Brickenden, A. M. & Sanwal, B. D. (1984a) *J. Biol. Chem.* **259**, 2646–2650
- Cates, G. A., Kaur, H. & Sanwal, B. D. (1984b) *Can. J. Biochem. Cell Biol.* **62**, 28–35
- Fuhrmann, U., Bause, E., Legler, G. & Ploegh, H. (1984) *Nature (London)* **307**, 755–758
- Gilfix, B. M. & Sanwal, B. D. (1980) *Biochem. Biophys. Res. Commun.* **96**, 1184–1191
- Gilfix, B. M. & Sanwal, B. D. (1984) *Can. J. Biochem. Cell Biol.* **62**, 60–71
- Gross, V., Andus, T., Tran-Thi, T.-A., Schwarz, R. T., Decker, K. & Heinrich, P. C. (1983) *J. Biol. Chem.* **258**, 12203–12209
- Hettkamp, H., Legler, G. & Bause, E. (1984) *Eur. J. Biochem.* **142**, 85–90
- Holland, P. C., Pena, S. D. J. & Guerin, C. W. (1984) *Biochem. J.* **218**, 465–473
- Kilker, R. D., Jr., Saunier, B., Tkacz, J. S. & Herscovics, A. (1981) *J. Biol. Chem.* **256**, 5299–5303
- Knudsen, K. A. (1985) *J. Cell Biol.* **101**, 891–897
- Knudsen, K. A. & Horwitz, A. F. (1977) *Dev. Biol.* **58**, 328–338
- Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664
- Lodish, H. F. & Kong, N. (1984) *J. Cell Biol.* **98**, 1720–1729
- Marino, M., Cossu, G., Neri, G. & Molinaro, M. (1980) *Dev. Biol.* **78**, 258–267
- Nichols, E. J., Manger, R., Hakomori, S., Herscovics, A. & Rohrschneider, L. R. (1985) *Mol. Cell. Biol.* **5**, 3467–3475
- Olden, K., Law, J., Hunter, V. A., Romain, R. & Parent, J. B. (1981) *J. Cell Biol.* **88**, 199–204
- Pan, Y. T., Hori, H., Saul, R., Sanford, B. A., Molyneux, R. J. & Elbein, A. D. (1983) *Biochemistry* **22**, 3975–3984
- Parfett, C. L. J., Jamieson, J. C. & Wright, J. A. (1983) *Exp. Cell Res.* **144**, 405–415
- Peyrieras, N., Bause, E., Legler, G., Vasilov, R., Claesson, L., Peterson, P. & Ploegh, H. (1983) *EMBO J.* **2**, 823–832
- Romero, P. A., Datema, R. & Schwarz, R. T. (1983) *Virology* **130**, 238–242
- Romero, P. A., Saunier, B. & Herscovics, A. (1985a) *Biochem. J.* **226**, 733–740
- Romero, P. A., Friedlander, P. & Herscovics, A. (1985b) *FEBS Lett.* **183**, 29–32
- Romero, P. A., Friedlander, P., Fellows, L., Evans, S. V. & Herscovics, A. (1985c) *FEBS Lett.* **184**, 197–201
- Saunier, B., Kilker, R. D. Jr., Tkacz, J. S., Quaroni, A. & Herscovics, A. (1982) *J. Biol. Chem.* **257**, 14155–14161
- Schwarz, R. T. & Datema, R. (1984) *Trends Biochem. Sci.* **9**, 32–34
- Wahrman, J. P., Senchal, H., Etienne-Decerf, J. & Winand, R. J. (1980) *FEBS Lett.* **115**, 230–234
- Walsh, F. & Phillips, E. (1981) *Dev. Biol.* **81**, 229–237
- Yaffe, D. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **61**, 477–483