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Cell interactions in preimplantation embryos: evidence for involvement of saccharides of the poly-N-acetyllactosamine series.

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SUMMARY

Roles of cell surface carbohydrates containing the 3-fucosyl-N-acetyllactosamine and poly-N-acetyllactosamine sequences (SSEA-1 and I antigens, respectively) in the compaction of mouse embryos have been investigated using the endo-β-galactosidase of *Bacteroides fragilis* to modify the surface of cleavage-stage embryos. Treatment with this enzyme abolished SSEA-1 activity and diminished I antigen activity on the embryonic cell surface. Embryos cultured in the presence of endo-β-galactosidase from the 2- to 4-cell stage onwards, or treated with the enzyme at the compacting 8-cell stage, continued to compact and proceeded to form blastocysts at the normal rate. However, when compacted 8- to 16-cell embryos were experimentally decompacted in calcium-free medium, treated for 1 h with endo-β-galactosidase and returned to normal culture medium, the time taken for 50 % of the embryos to recompact was prolonged five-fold. There was an even greater delay if these embryos were maintained in culture medium containing the enzyme. Blastocysts were eventually formed under both conditions. Thus, endo-β-galactosidase did not affect compaction unless the embryos were first decompacted. On the assumption that recompaction and *de novo* compaction occur by similar mechanisms, we propose that carbohydrate-binding molecules are involved which have high affinities for poly-N-acetyllactosamine structures and protect them from digestion by endo-β-galactosidase.

INTRODUCTION

Compaction is a complex process during which polarization of blastomeres occurs, cell–cell contacts are maximized and specialized junctions formed (Handyside, 1980; Reeve & Ziomek, 1981; Johnson, Pratt & Handyside, 1981). It is the first major morphological event in the development of the mammalian embryo, and in the mouse it takes place at the 8-cell stage (Ducibella & Anderson, 1975). Compaction is a prerequisite for blastocyst formation. The precise mechanism is not yet known but interactions between cell surface molecules and the cytoskeleton are thought to be involved (Ducibella & Anderson, 1975; Surani, Barton & Burling 1980; Sutherland & Calarco-Gillam, 1983) and there is a requirement for

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calcium ions (Ducibella & Anderson, 1979). Immunological studies have revealed calcium-dependent adhesive glycoproteins (Hyafil, Morello, Babinet & Jacob, 1980; Hyafil, Babinet & Jacob, 1981; Takeichi 1981; Yoshida & Takeichi, 1982; Ogou, Yoshida-Noro & Takeichi 1982; Shirayoshi, Okada & Takeichi, 1983; Peyrieras *et al.* 1983; Yoshida-Noro, Suzuki & Takeichi, 1984) which participate in the compaction process, but the precise determinants that mediate the adhesion have not yet been characterized.

There is evidence that carbohydrate structures may have important roles in early embryonic cell interactions. It has been reported that compaction is inhibited and reversed in the presence of inhibitors of protein N-glycosylation (Surani, 1979; Surani, Kimber & Handyside, 1981; Atienza-Samols, Pine & Sherman, 1981; Sutherland & Calarco-Gillam, 1983). A cell surface galactosyltransferase activity has been detected on embryonal carcinoma cells of the mouse and evidence presented that this enzyme may mediate cell–cell adherence by bridge formation with pericellularly located acceptor substrates that are susceptible to digestion with endo-β-galactosidase (Shur, 1982, 1983). Moreover, there is immunochemical evidence that a cell surface galactosyltransferase appears in the mouse embryo at the late 8-cell stage (Sato, Muramatsu & Berger, 1984).

Endo-β-galactosidase is known to cleave specifically the internal β-galactosidic linkages of oligosaccharides of the poly-N-acetyllactosamine series with the sequence (Galβ1-4GlcNAcβ1-3)$_n$ (Fukuda & Matsumura, 1976). Saccharides of this series are abundant on the surface of cleavage-stage mouse embryos (Muramatsu *et al.* 1978). Studies with various monoclonal anti-carbohydrate antibodies (Kapadia, Feizi & Evans, 1981; Gooi *et al.* 1981) have indicated that during embryonic development, the structures of these saccharides change in a highly ordered and stage-specific manner consistent with (a) the presence of branched backbone sequences throughout the first seven days of gestation (I antigenic determinants, such as those expressed on Structures 1 and 2, Table 1), (b) the onset of α1-3 fucosylation of N-acetylglucosamine residues at the 8-cell stage [forming the stage-specific embryonic antigen, SSEA-1 (Solter & Knowles, 1978), Structure 4] and (c) the appearance of long, unbranched backbone structures (i antigen, Structure 3) on the surface of primary endoderm cells, the first differentiated cells that can be discerned on the inner cell mass. It has been suggested that these carbohydrate sequences, or the substitutions that they bear, may be recognition structures which determine the cellular orientation, migration and responses to regulatory factors during development (Kapadia *et al.* 1981; Gooi *et al.* 1981 Feizi, 1982). In particular it has been suggested that the SSEA-1 structure, which is first detectable at the 8-cell stage, may have a role in compaction by a mechanism which involves interaction with an endogenous lectin.

In the present study we have used the endo-β-galactosidase of *B. fragilis* to modify the surface of cleavage-stage embryos and to investigate directly the role of cell surface oligosaccharides based on poly-N-acetyllactosamine structures in the events leading to compaction of mouse embryos.

MATERIALS AND METHODS

Embryos and culture procedures

Six- to eight-week-old (C57Bl/10 × CBA)F1 female mice were superovulated with 5 i.u. pregnant mare serum gonadotrophin (Intervet, Science Park, Middleton, Cambridge, UK.) followed, 44 h later, by 5 i.u. human chorionic gonadotrophin (Intervet). They were mated with (C57B1/10 × CBA)F1 male mice and, on the following morning, checked for the presence of copulation plugs. Ovulation was assumed to occur 12 h after HCG injection. Embryos were flushed from the oviducts at the 2- to 4-cell stage, 48–52 h after HCG, using a Hepes buffered medium M2 (Quinn, Barros & Whittingham, 1982) containing 4 mg bovine serum albumin (BSA) ml^{-1} (referred to as M2). Embryos were cultured in microdrops of medium M16 (Whittingham, 1971) containing 4 mg BSA ml^{-1} (referred to as M16) overlaid with paraffin oil in Falcon tissue culture dishes in a humidified atmosphere of 5 % CO_2 in air at 37 °C. Manipulations of embryos were carried out in Hepes buffered M2 medium under a Wild dissecting microscope. Zonae pellucidae were removed by treating fixed or unfixed embryos with a 5 $mg.ml^{-1}$ solution of Pronase (Calbiochem-Behring, La Jolla, California, USA) in complete phosphate-buffered saline (Dulbecco A + B) containing 10 $mg.ml^{-1}$ polyvinyl pyrrolidine (Calbiochem-Behring) for 5–10 minutes at 37 °C.

Endo-β-galactosidase

Endo-β-galactosidase, free from any detectable exoglycosidase activity, was isolated from culture filtrates of *Bacteroides fragilis* as described previously (Scudder *et al.* 1983a). An assay for proteinase activity in which one unit of endo-β-galactosidase ml^{-1} was incubated with various proteins (2 $mg.ml^{-1}$) followed by electrophoresis in SDS/polyacrylamide gels and silver staining (Morrisey, 1981) for protein bands, failed to detect any hydrolysis products; the enzyme preparation was therefore judged to be free from proteinase activity. Lyophilized enzyme was dissolved in M16 or calcium-free M16 to give an activity of 0.5 $units.ml^{-1}$.

Endo-β-galactosidase treatment of embryos

The effect of endo-β-galactosidase treatment of embryos on compaction and subsequent development to blastocysts was studied in three ways.

(a) 2- to 4-cell embryos were cultured in M16 containing endo-β-galactosidase.

(b) compacting 8-cell embryos were cultured in M16 containing endo-β-galactosidase.

(c) compacted 8- to 16-cell embryos both with and without their zonae pellucidae, were experimentally decompacted by incubation in calcium-free M2 at 37 °C for 1 h and then treated with endo-β-galactosidase in calcium-free M16 for 1 h. Embryos were then transferred into normal M16 or M16 containing endo-β-galactosidase for continued culture.

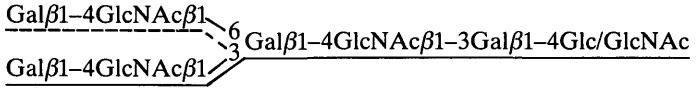
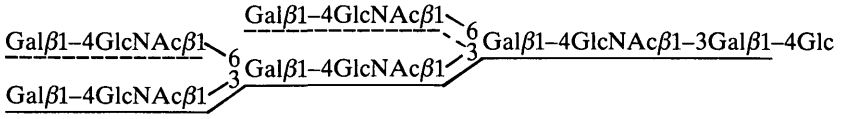
In each experiment, control embryos were subjected to identical procedures except that endo-β-galactosidase was omitted. In some experiments, enzyme which had been inactivated by heating at 100 °C for 3 minutes was used as an additional control. Each experiment was repeated at least three times with a minimum of 250 embryos, except experiment (c), in the absence of zonae pellucidae, which was performed once with 160 embryos.

At the end of each experiment endo-β-galactosidase was still active as judged by its ability to completely abolish haemagglutination of erythrocytes of i antigen type with a monoclonal anti-i antibody.

Monoclonal antibodies

Anti-I Ma and anti-I Step are naturally occurring monoclonal antibodies in sera of patients with cold agglutinin disease (Feizi, 1981). They recognize epitopes on branched carbohydrate chains of poly-N-acetyllactosamine type such as Structures 1 and 2 (Table 1). Anti-SSEA-1 recognizes the 3-fucosyl-N-acetyllactosamine sequence, Structure 4. (Gooi *et al.* 1981; Hounsell *et al.* 1981). This may occur as a terminal structure on carbohydrate sequences such as 1, 2 and 3 on glycoproteins and glycolipids. It also occurs in soluble form on the human milk oligosaccharide, lacto-N-fucopentaose III (LNF III, Structure 5). Anti-SSEA-1 is strongly inhibited by 3-fucosyl-

Table 1. *Carbohydrate structures cited in the text*

	1*
	2*
Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc/GlcNAc	3
Galβ1–4GlcNAc | 1, 3 Fucα	4
Galβ1–4GlcNAcβ1–3Galβ1–4Glc | 1, 3 Fucα	5
Galβ1–3GlcNAcβ1–3Galβ1–4Glc | 1, 4 Fucα	6
Galβ1–4Glc | 1, 3 Fucα	7
Galβ1–4Glc	8
Galβ1–4GlcNAc	9

* In Structures 1 and 2 the sequence recognized by anti-I Ma is shown by dotted underlining and those reactive with anti-I Step, by solid underlining.

N-acetyllactosamine and LNF III; it is weakly inhibited by the milk oligosaccharide 3-fucosyllactose, Structure 7, but not at all by the milk oligosaccharide lacto-N-fucopentaose II (LNF 11), Structure 6, in which the terminal trisaccharide sequence is the blood group Le^a determinant. Anti-I antibodies were used as serum (anti-I Ma 1:100 dilution; anti-I Step, 1:700) and anti-SSEA-1 as ascites (1:100). Normal human serum supplemented with IgM (Feizi *et al.*, 1980) and normal mouse serum were used as negative controls.

Immunofluorescence microscopy

Embryos were fixed in 4 % (w/v) formaldehyde in PBS (Dulbecco A+B for compacted embryos; Dulbecco A for decompacted embryos) for 1 h at room temperature, then washed three times in M2. After removal of zonae pellucidae with Pronase and washing three times in M2, the embryos were incubated at 4 °C in anti-SSEA-1 or normal mouse serum followed by fluorescein-labelled rabbit anti-mouse immunoglobulins (Nordic Immunological Laboratories, Maidenhead, Berks, UK), or in anti-I Ma or anti-I Step or normal human serum followed by fluorescein-labelled rabbit anti-human IgM (Dako Immunoglobulins, Mercia Brocades, Weybridge, Surrey, UK). Each incubation was for 30 minutes and unbound antibodies were removed by washing in

three changes of M2. All manipulations were carried out in 100 μl drops of the appropriate solution. Embryos were then mounted in glycerol containing 4% (w/v) formaldehyde and 0·22 M 1,4-diazabicyclo[2,2,2]octane (Sigma Chemical Co., Poole, Dorset UK), pH 8·6 (Johnson *et al.* 1982) and viewed using a Zeiss epifluorescence microscope. The intensity of immunofluorescence above control staining was graded from weak to very strong. In preliminary experiments, treatment of embryos with Pronase for up to 15 minutes did not affect their immunofluorescence reactions with the reagents used in this study. Fluorescence and phase-contrast photomicrographs were taken using Ilford HP5 and Panatomic X films respectively.

RESULTS

Effect of culturing 2- to 4-cell embryos in the presence of endo-β-galactosidase

2- to 4-cell embryos cultured continuously in the presence of endo-β-galactosidase compacted normally between 70 and 74 h after HCG injection, and, by 120 h post HCG, they formed expanded blastocysts with inner cell masses (Fig. 1A). Although the enzyme-treated embryos were morphologically indistinguishable from the controls, the embryos cultured in this enzyme showed no immunofluorescence with anti-SSEA-1 at the compacting 8-cell stage, whereas the control embryos showed a moderately strong immunofluorescence (Fig. 2). SSEA-1

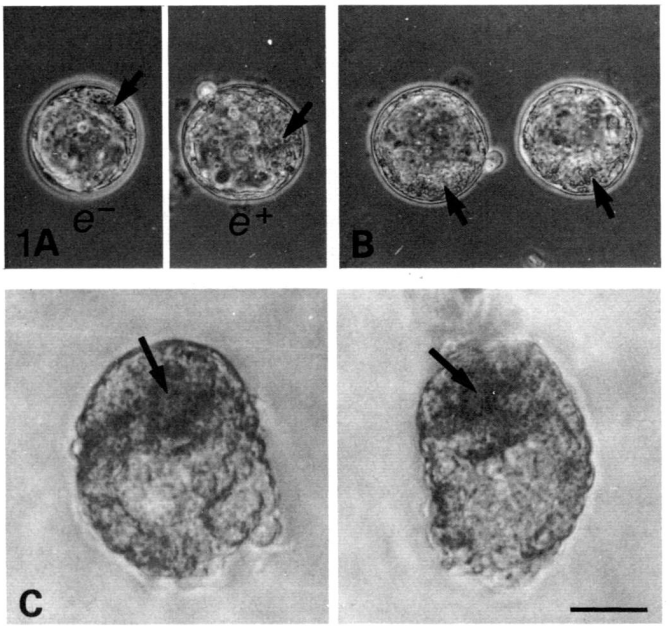


Fig. 1. Phase contrast photomicrographs showing morphologically normal expanded blastocysts; (A), derived from embryos cultured from the 2- to 4-cell stage onwards in the presence (e^+) or absence (e^-) of endo-β-galactosidase; (B), derived from experimentally decompacted 8-cell embryos treated with endo-β-galactosidase, and recompacted under Condition 2; (C), giant blastocysts formed from zona-free, experimentally decompacted 8-cell embryos treated with endo-β-galactosidase, which reaggregated and recompacted under Condition 2. Arrows indicate the inner cell mass. Bar represents 65 μm.

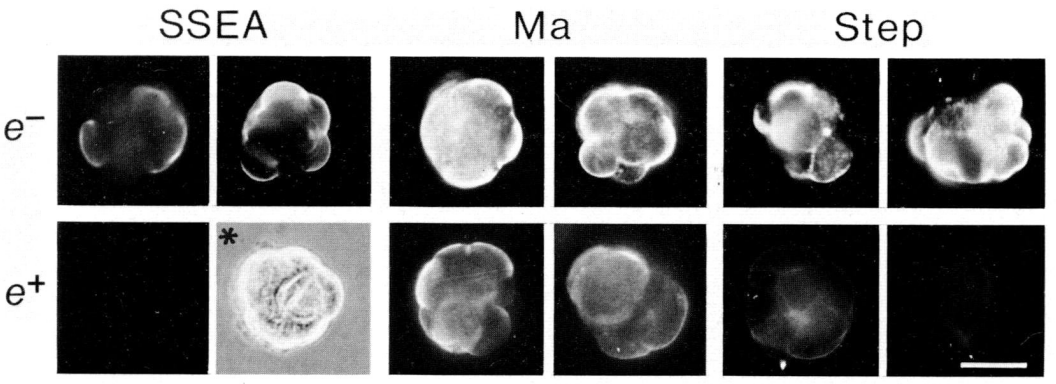


Fig. 2. Immunofluorescence with anti-SSEA-1, anti-I Ma and anti-I Step of compacted 8-cell embryos which had been cultured in the presence (e^+) or absence (e^-) of endo-β-galactosidase from the 2- to 4-cell stage. In e^+ with anti-SSEA-1, the corresponding phase-contrast micrograph of an antigen-negative embryo is shown (indicated with an asterisk). Bar represents 50 μm.

reactivity was not revealed at contact sites of the compacted, enzyme-treated embryos that were decompacted by calcium depletion during fixation prior to immunofluorescence (results not shown). Immunofluorescence with anti-I Ma and anti-I Step was reduced but not abolished in enzyme-treated embryos (Fig. 2).

Effect of endo-β-galactosidase on compacting 8-cell embryos

Incubation of compacting 8-cell embryos in medium containing endo-β-galactosidase did not cause decompaction. Furthermore, embryos cultured continuously from the compacted 8- to 16-cell stage onwards in medium containing endo-β-galactosidase developed at the same rate as the controls to form expanded blastocysts at 120 h after HCG injection. Again, SSEA-1 expression was abolished and I(Ma) and I(Step) expression was reduced in the enzyme treated embryos (results not shown).

Effect of endo-β-galactosidase on experimentally decompacted 8-cell embryos

When 8- to 16-cell embryos which had been experimentally decompacted by calcium depletion were treated with endo-β-galactosidase in calcium-free medium for 1 h, and then returned to normal medium (Condition 1), the time taken for recompaction of 50 % of embryos was 190 mins, compared with 36 mins for control embryos which had been incubated only in calcium-free medium or in calcium-free medium containing heat-inactivated enzyme (Figure 3). With embryos which had been treated with endo-β-galactosidase as above and returned to normal calcium-containing medium with endo-β-galactosidase (Condition 2), recompaction was even further delayed, but was complete by 16 h. By 120 h after HCG, embryos under Conditions 1 and 2 formed morphologically normal expanded blastocysts (Figure 1B). Immunofluorescence, carried out immediately after enzyme treatment

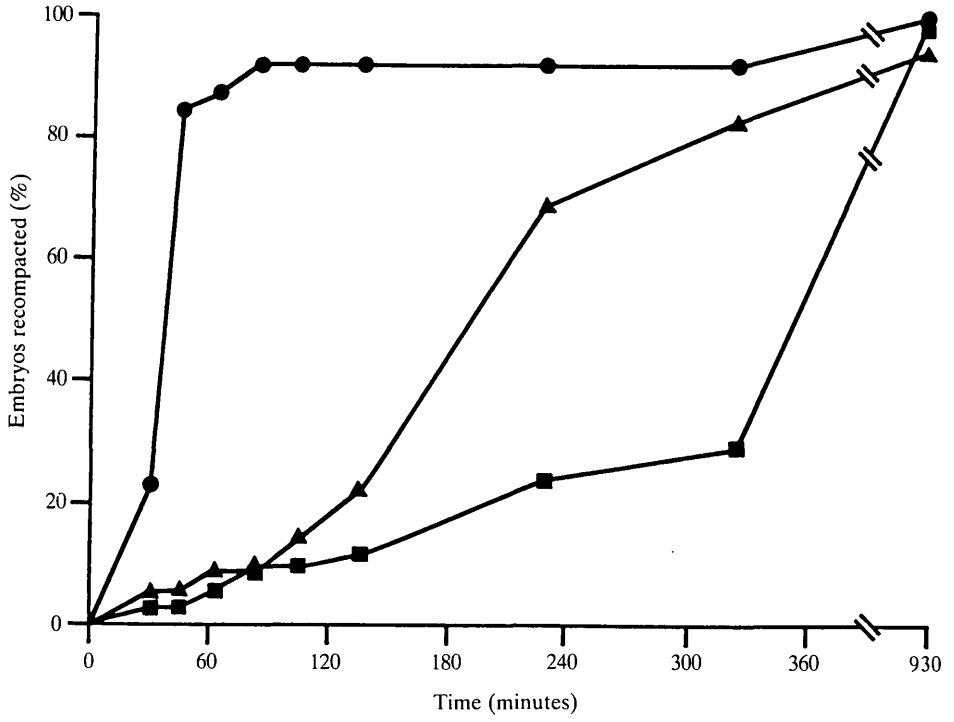


Fig. 3. Graph showing the time taken for recompaction to occur in experimentally decompacted 8-cell embryos which were treated with endo-β-galactosidase for 1 h and then allowed to recompact in normal M16, Condition 1, (▲) or in M16 containing endo-β-galactosidase, Condition 2, (■). Control embryos (●) were experimentally decompacted and cultured for 1 h in M16 containing heat-inactivated enzyme followed by culture in normal M16. Each point represents a minimum of 33 embryos.

showed that SSEA-1 expression had been completely abolished, whereas there was a moderately strong staining of this antigen in control embryos (Fig. 4A). At recompaction, SSEA-1 had reappeared on embryos which had been treated with endo-β-galactosidase under Condition 1, although the intensity of immunofluorescence was weaker than that of control embryos (Fig. 4B). However, this antigen was not detectable on embryos that had eventually compacted in endo-β-galactosidase-containing medium under Condition 2 (Fig. 4C). The I(Ma) and I(Step) immunofluorescence was slightly diminished immediately after enzyme treatment (Fig. 4A) but appeared as intense as in control embryos at recompaction under Condition 1 (Fig. 4B). Both antigenic determinants were reduced on the embryos that recompacted in the presence of endo-β-galactosidase under Condition 2 (Fig. 4C).

Experiments were repeated using compacted 8- to 16-cell embryos from which zonae pellucidae were removed prior to decompaction. In calcium-free medium, the embryos became dissociated into individual blastomeres. Upon return to calcium-containing medium, control blastomeres and those treated with endo-β-

galactosidase under Conditions 1 and 2 aggregated into large masses. The embryos were examined at 30- to 60-min intervals. However, the onset of compaction was difficult to assess with precision. By 100 mins, the control blastomeres appeared completely compacted, whereas blastomeres treated with endo-β-galactosidase under both Conditions 1 and 2, recompacted at the same rate as

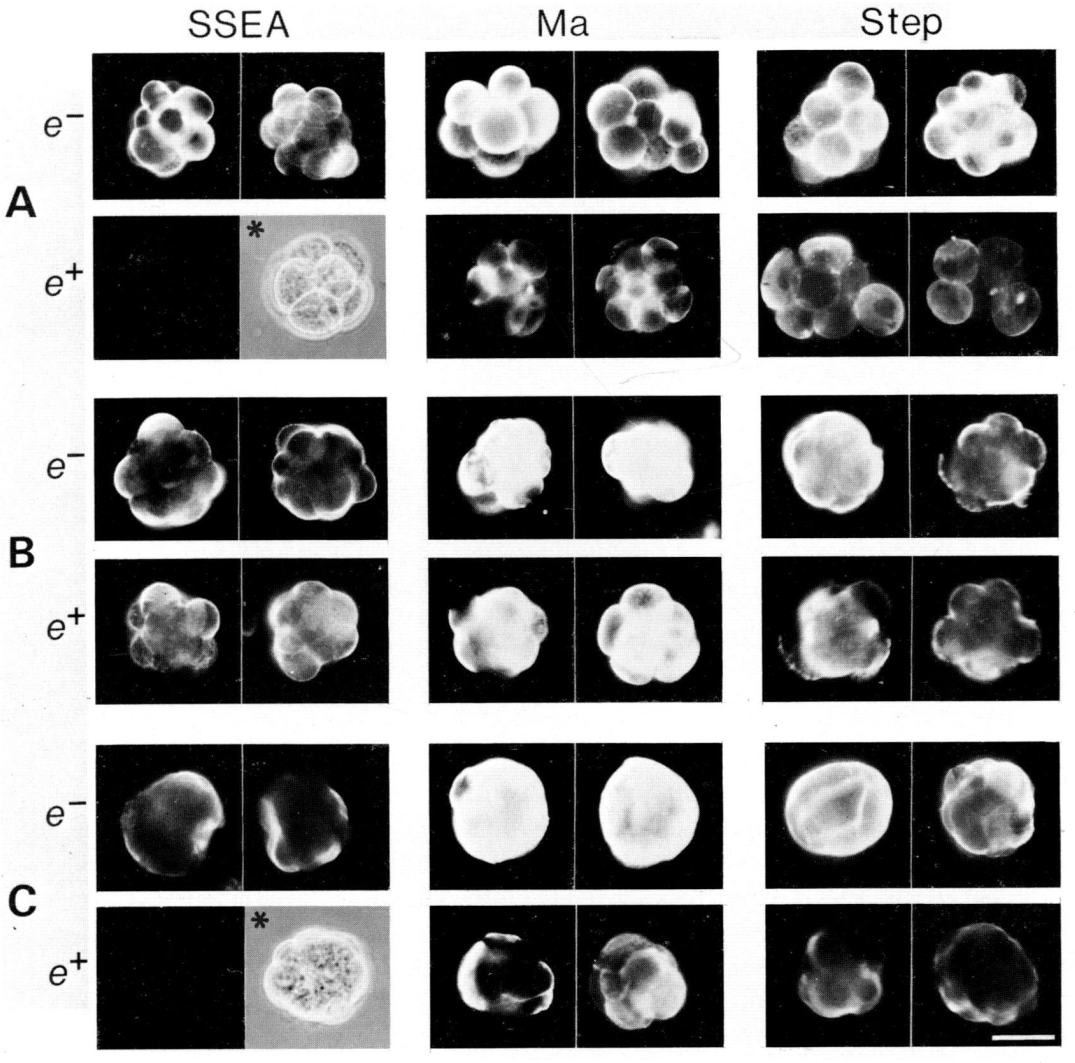


Fig. 4. Immunofluorescence with anti-SSEA-1, anti I-Ma and anti I Step of experimentally decompacted 8-cell embryos which had been incubated for 1 h in the presence (e^+) or absence (e^-) of endo-β-galactosidase: immunofluorescence was carried out in (A), immediately after enzyme treatment; in (B), when the enzyme-treated embryos had recompacted in M16 under Condition 1; and in (C), when the enzyme-treated embryos had recompacted in M16 containing enzyme under Condition 2. In Ae^+ and Ce^+ with anti-SSEA-1, the corresponding phase-contrast micrographs of antigen-negative embryos are shown (indicated with asterisks).

each other and were fully compacted by 260 mins. This corresponded to the compaction time of zona-bearing embryos under Condition 1. The controls and the two enzyme-treated groups formed giant blastocysts by 120 h after HCG injection (Fig. 1C).

DISCUSSION

Although carbohydrate structures of the cell surface are generally assumed to be involved in cell–cell interactions during mammalian development and differentiation, there has been no direct evidence for a recognition role for any particular carbohydrate sequence. This may be due to the simultaneous involvement of multiple recognition systems. The cleavage-stage mouse embryo seems an ideal model for investigation on account of the information available on the carbohydrate antigens/structures on the blastomere surface.

Our approach has been to modify the blastomere surface using endo-β-galactosidase. A delay in compaction was only observed with compacted 8- to 16-cell embryos which were experimentally decompacted and allowed to recompact after enzyme treatment, suggesting that carbohydrate chains cleaved by the enzyme are involved in the recompaction process.

Available information on the specificity of this enzyme is summarized in Table 2. In brief, internal Galβ1-4/3GlcNAc/Glc linkages are cleaved when they are part of linear domains. Branched domains are not usually cleaved from their carrier macromolecules unless there are intervening linear sequences as in Structure b, c and e. Other factors which confer resistance (reviewed by Scudder, Hanfland, Uemura & Feizi, 1984) include sulphation of the galactose residues and fucosylation of N-acetylglucosamine residues in close proximity to the susceptible β-galactosidase linkage. In the present studies, the loss of SSEA-1 activity following treatment with endo-β-galactosidase suggests that, as with embryonal carcinoma cells of the mouse (Childs *et al.* 1983), the majority of these determinants are borne on oligosaccharides containing linear poly-N-acetyllactosamine domains. In accordance with previous observations with mucin-type glycoproteins (Scudder *et al.* 1983b) I antigen activities associated with branched poly-N-acetyllactosamine sequences, were diminished but not abolished on embryos treated with this enzyme.

Although, the presence of small amounts of residual SSEA-1 determinants cannot be ruled out by immunofluorescence, our studies have shown that compaction and subsequent development to the expanded blastocyst can take place in the absence of serologically detectable SSEA-1. These observations are in accord with those of Rosenstraus (1983) who described an embryonal carcinoma cell line which lacks this antigen and yet shows normal cell interaction and developmental properties. Furthermore, the partial removal of poly-N-acetyllactosamine sequences with endo-β-galactosidase is compatible with the onset and completion of compaction at a normal rate. However, some role for carbohydrate structures in the recompaction

Table 2. *Summary of information on the susceptibilities of unsubstituted linear and branched poly-N-acetyllactosamine sequences to endo-β-galactosidase*

The specificities shown here are deduced from studies using endo-β-galactosidases from *B. fragilis* and/or *E. freundii*; these enzymes have almost identical properties (Scudder *et al.* 1983a and 1984).

	Structures	References
a	Galβ1–4GlcNAcβ1–3Galβ1$^{S}\downarrow$4GlcNAcβ1–3Galβ1$^{S}\downarrow$4Glc/GlcNAc...	Fukuda, Fukuda & Hakomori, 1979
b	Galβ1–4GlcNAcβ1$\diagdown^{6}$ Galβ1–4GlcNAcβ1$\diagup_{3}$Galβ1$^{R}\downarrow$4GlcNAcβ1–3Galβ1$^{S}\downarrow$4Glcβ1–Ceramide	Scudder *et al.* 1984
c	Galβ1–4GlcNAcβ1$\diagdown^{6}$ Galβ1–4GlcNAcβ1$\diagdown^{6}$ Galβ1–4GlcNAcβ1$\diagup_{3}$Galβ1$^{R}\downarrow$4GlcNAcβ1$\diagup_{3}$Galβ1$^{R}\downarrow$4GlcNAcβ1–3Galβ1$^{S}\downarrow$4Glcβ1–Ceramide	Scudder *et al.* 1984
d	Galβ1–4GlcNAcβ1$\diagdown^{6}$ Galβ1–4GlcNAcβ1$\diagup_{3}$Galβ1$^{R}\downarrow$4GlcNAcβ1–3Galβ1$^{R}\downarrow$4GlcNAcβ1–2Manα1$\diagdown_{3}$Man... ...Manα1$\diagup^{6}$	Fukuda, Dell, Oates & Fukuda, 1984
e	Galβ1–4GlcNAcβ1$\diagdown^{6}$ Galβ1–4GlcNAcβ1$\diagup_{3}$Galβ1$^{S}\downarrow$4GlcNAcβ1–3Galβ1$^{S}\downarrow$4GlcNAcβ1–3Galβ1$^{S}\downarrow$4GlcNAcβ1–2Manα1$\diagdown_{3}$Man... ...Manα1$\diagup^{6}$	Fukuda *et al.* 1984

process is suggested by the pronounced delay in recompaction of experimentally decompacted embryos that had been treated with endo-β-galactosidase.

Our attempts to identify precise carbohydrate sequences involved in compaction have been unsuccessful thus far. In unpublished experiments we have not observed an inhibition, delay or reversal of compaction of embryos grown from the 2- to 4-cell stage or the experimentally decompacted 8-cell stage in the presence of 1–10 $mg.ml^{-1}$ concentrations of 3-fucosyl-N-acetyllactosamine (Structure 4) or 0·1–1 $mg.ml^{-1}$ of various blood group glycoproteins (Gooi *et al.* 1981) which express strong SSEA-1, I and i antigen activities. Lactose (Structure 8), N-acetyllactosamine (Structure 9), and the above mentioned glycoproteins treated with mild acid (Gooi *et al.* 1983) to remove fucose and sialic acid residues and expose galactose residues, also had no effect on compaction. This suggests that these glycans do not have optimal structures or valency for effective competition with those on the embryo. The only effect of the oligosaccharides (5–10 $mg.ml^{-1}$) on embryonic development was a marked reduction in blastocyst formation. This was observed in 2- to 4-cell embryos (10–20 % blastocyst formation, compared with 70% for controls) and experimentally decompacted 8-cell embryos (34–38 % blastocyst formation, compared with 90% for controls). These effects may be attributable to a toxicity of the high concentrations of the oligosaccharides. Our observations contrast with those of Bird & Kimber (1984) who noted a reversal of compaction of 8- to 16-cell mouse embryos using two analogues of 3-fucosyl-N-acetyllactosamine, namely, 3-fucosyllactose (Structure 7) and lacto-N-fucopentaose III (Structure 5), as well as the Lewis[a] active oligosaccharide, lacto-N-fucopentaose II (Structure 6).

In conclusion, we have shown that compaction and blastocyst formation of mouse embryos can take place in the presence of endo-β-galactosidase which removes serologically detectable SSEA-1 antigen from the surface of blastomeres and reduces their I antigen activity. An effect of the enzyme on compaction could only be shown after experimental decompaction of the early compacting embryos. This could be interpreted to mean that the enzyme-susceptible saccharides are not involved in natural compaction. However, on the assumption that the processes operating during recompaction are similar to those occuring during natural compaction, we consider it more likely that they are mediated by adhesive molecules with such high affinities for poly-N-acetyllactosamine-borne sequences that they protect the normally susceptible structures from degradation by endo-β-galactosidase. Further investigations are required to establish the roles of specific saccharide structures, glycosyltransferases or other carbohydrate-binding proteins as mediators of adhesion during the compaction process, and, the time is ripe for investigating whether the various immunochemically characterized surface adhesins of the mouse embryo are carbohydrate-binding proteins.

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ADDENDUM

At submission stage we were informed that Fenderson, Zehavi and Hakomori (J. exp. Med. **160**, 1591–1598, (1984)) have observed decompaction of 8- to 16-cell mouse embryos with a trivalent conjugate of lacto-N-fucopentaose III but not with the free oligosaccharide nor with the trivalent conjugate of the oligosaccharide isomer lacto-N-fucopentaose II.

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