Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 728–732, January 1994
Medical Sciences

Mice lacking *N*-acetylglucosaminyltransferase I activity die at mid-gestation, revealing an essential role for complex or hybrid N-linked carbohydrates

(glycobiology/homologous recombination/glycosyltransferase gene/mouse development)

ELLA IOFFE AND PAMELA STANLEY*

Department of Cell Biology, Albert Einstein College of Medicine, New York, NY 10461

Communicated by Salome G. Waelsch, October 13, 1993

ABSTRACT **Eukaryotic cells require N-linked carbohydrates for survival. However, the biosynthetic intermediate Man5GlcNAc2Asn, in place of mature N-linked structures, allows glycoprotein synthesis and somatic cell growth to proceed normally. To determine whether the same would be true in a complex biological situation, the gene *Mgat-1* was disrupted by homologous recombination in embryonic stem cells and transmitted to the germ line. The *Mgat-1* gene encodes *N*-acetylglucosaminyltransferase I [GlcNAc-TI; α-1,3-mannosyl-glycoprotein β-1,2-*N*-acetylglucosaminyltransferase; UDP-*N*-acetyl-D-glucosamine:glycoprotein (*N*-acetyl-D-glucosamine to α-D-mannosyl-1,3-(R_1)-β-D-mannosyl-R_2) β-1,2-*N*-acetyl-D-glucosaminyltransferase, EC 2.4.1.101], the transferase that initiates synthesis of hybrid and complex N-linked carbohydrates from Man5GlcNAc2Asn. Mice lacking GlcNAc-TI activity did not survive to term. Biochemical and morphological analyses of embryos from 8.5 to 13.5 days of gestation showed that *Mgat-1*$^{-/-}$ embryos are developmentally retarded, most noticeably in neural tissue, and die between 9.5 and 10.5 days of development.**

The mammalian carbohydrates associated with glycoconjugates have many biological functions (1). However, defining specific roles for carbohydrates is complicated because identical sugar arrangements are found on unrelated glycolipids and glycoproteins. For example, the developmentally regulated tetrasaccharide sialylated Le^x (SLe^x) {NeuNAc(α 2-3) Gal(β 1-4)[Fuc(α 1-3)]GlcNAcβ1} is found on N- and O-linked carbohydrates of glycoproteins (2, 3) and on glycolipids (4). This carbohydrate is important in recognition events mediated by the selectins (5). Although physiological selectin ligands probably express SLe^x on O-linked carbohydrates (6–8), N-linked carbohydrates bearing SLe^x on LEC11 Chinese hamster ovary (CHO) cells are able to mediate E-selectin binding (9, 10). Thus, in a complex organism, redundant mechanisms may exist to ensure expression of carbohydrates important for survival.

One approach to investigating the flexibility of an organism to generate biologically important carbohydrates is to eliminate the synthesis of one class of carbohydrates. This experiment became possible in mammals with the cloning of the mouse gene *Mgat-1* (11, 12) that encodes *N*-acetylglucosaminyltransferase I (GlcNAc-TI, EC 2.4.1.101). This transferase is essential for the conversion of Man5GlcNAc2Asn to complex or hybrid N-linked carbohydrates (13). It was discovered as the transferase missing in mutant CHO cells resistant to plant lectins (14, 15). These mutants proved that GlcNAc-TI is not required for viability or appropriate glycoprotein compartmentalization in somatic cells (16). Mutants of baby hamster kidney (BHK) cells that can make hybrid but not complex N-linked carbohydrates are also viable (17, 18). In humans, the reduced ability to synthesize complex N-linked carbohydrates has debilitating but not lethal effects (19, 20). To investigate the consequences of abrogating both complex and hybrid moieties in a developing organism, we have disrupted the *Mgat-1* gene by homologous recombination in embryonic stem (ES) cells. Mice homozygous for a null mutation at this locus die at mid-gestation, revealing an absolute requirement for N-linked complex or hybrid carbohydrates for development beyond this stage.

MATERIALS AND METHODS

Selection of Homologous Recombinants. The ES cell line WW6 was generated by F. Poirier and E. J. Robertson (Harvard University) from the inner cell mass of a blastocyst from a mouse (50% 129/Sv; 25% C57BL/6; 5% SJL; 20% unknown) that carries a large tandem repeat of the mouse β-globin gene as a transgene (21). WW6 cells were cultured on feeder cells in alpha medium (GIBCO) containing 10% fetal bovine serum (GIBCO) and 20 μg of gentamicin per ml (GIBCO). The feeder cells (SNL2; developed by E. J. Robertson) were γ-irradiated, G418-resistant STO fibroblasts that express a transfected leukemia inhibitory factor gene.

The targeting vector SXNeoTK7 was constructed from a 3.8-kb *Sac* I fragment of the *Mgat-1* gene (12) in pGEM.7Zf (+). A neomycin-resistance (neo) gene under control of the phosphoglycerate kinase (pgk) promoter and with a pgk poly(A) addition site was inserted in the reverse orientation at the *Xma* III site of the *Mgat-1* coding region (Fig. 1). A herpes simplex virus thymidine kinase (HSV-TK) gene also with a pgk promoter and a pgk poly(A) site was inserted at the *Eco*RI/*Hin*dIII sites of the vector 5′ of the *Sac* I fragment. Both cassettes were gifts of R. Jaenisch (Whitehead Institute) (22).

Targeting vector DNA (≈25 μg) linearized with *Hin*dIII was mixed with ≈2.0 × 10^7 WW6 cells at 4°C in 0.8 ml of phosphate-buffered saline (PBS) before electroporation in a gene pulser (Bio-Rad) at 960 μF and 220 V. Cells (10^7) were selected in medium containing active G418 (200 μg/ml) (GIBCO) and 2 μM ganciclovir (Syntex, Palo Alto, CA) or G418 alone. ES cell lines that had undergone mitotic recombination to generate two disrupted *Mgat-1* alleles (23) were selected for survival in active G418 (1.5 mg/ml) from homologous recombinant cell populations.

Screening of Recombinants and Generation of Mutant Mice. Genomic DNAs prepared from ES cells or mice tails were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GlcNAc-TI, *N*-acetylglucosaminyltransferase I; L-PHA, *Phaseolus vulgaris* leukoagglutinin; neo, neomycin-resistance gene; HSV-TK, herpes simplex virus thymidine kinase gene; β(1,4)Gal-T, β-1,4-galactosyltransferase; LIF, leukemia inhibitory factor; ES cell, embryonic stem cell; pgk, phosphoglycerate kinase; dpc, days postcoitus.

*To whom reprint requests should be addressed.

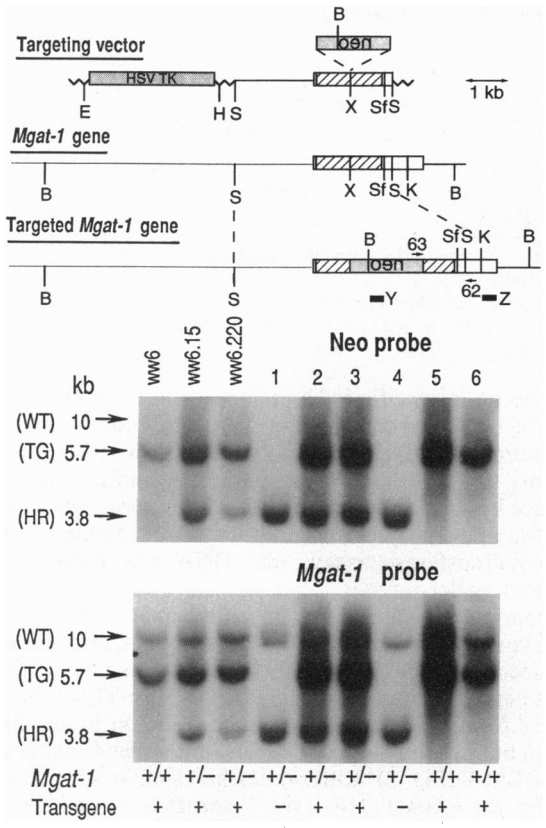

FIG. 1. Homologous recombination at the *Mgat-1* locus. Gene targeting strategy is shown in the diagram. *Mgat-1* gene open reading frame (hatched box), coding exon (open box), intron sequence (thin line), and vector sequences (broken line) are identified. Direction of transcription of the neo and HSV-TK genes is shown by label orientation. Disrupted *Mgat-1* gene is shown with primers 62 and 63 and probes Y (neo gene) and Z (*Mgat-1* gene). B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; S, *Sac* I; Sf, *Sfi* I; X, *Xma* III. (*Lower*) Southern blot of *Bam*HI-digested genomic DNAs from WW6 ES cells, two recombinants, and mice tails hybridized to probe Y (*Top*) or probe Z (*Bottom*). Lanes 1–6, DNA from progeny of a cross between a WW6.15-derived chimeric male and a C57BL/6 female. *Bam*HI fragments diagnostic for wild type (WT), homologous recombinant (HR), or transgene (TG) are indicated.

amplified in a final vol of 50 μl in PCR buffer (Boehringer Mannheim) containing 200 μM each dNTP, 2.5 mM $MgCl_2$, 0.4 μM each primer, and 50 units of *Taq* polymerase per ml (Boehringer Mannheim). Amplification was carried out for 40 cycles of 60 sec at 94°C, annealing for 2 min at 60°C, and extension for 3 min at 72°C, with a final extension step of 15 min. Oligonucleotide 62 (CTGCTCTGCTCCAGGACAAG) is the antisense sequence from nt 2271–2290 of a mouse *Mgat-1* cDNA (12); oligonucleotide 63 (GGTGGATGTGGAATGTGTGC) is the antisense sequence from the pgk promoter (nt −403 to −422) within the neo cassette (24). The ≈1.4-kb fragment generated from this primer pair was unique to targeted clones and specifically hybridized to a 349-bp *Pst* I fragment of the coding exon (12).

ES cells WW6.15 or WW6.220 were injected into blastocysts from C57BL/6 mice and transferred to pseudopregnant females as described (25). Chimeric males were bred to C57BL/6 or CD1 females and germ-line transmission was detected by PCR and confirmed by Southern blot analysis of tail DNA. Mutant mice homozygous for each *Mgat-1* gene disruption were obtained from heterozygote matings.

Southern and Northern Blot Analyses. Genomic DNAs (≤20 μg) from ES cells, mouse tails, yolk sacs, or embryos were digested with *Bam*HI and electrophoresed through a ≤0.8% agarose gel, transferred to Hybond-N membrane (Amersham) and hybridized at 42°C to probe Y or Z (Fig. 1) by standard methods (26). Probe Y is a 0.35-kb *Sph* I/*Bam*HI fragment derived from the neo cassette. Probe Z is an ≈0.4-kb exonuclease III fragment from the 3′ end of *Mgat-1* cDNA (12). Blots were washed and exposed to film at −70°C as described (26).

Total RNA was precipitated with LiCl (26) from ES cells, electrophoresed in a denaturing 1.5% agarose gel, and transferred to Hybond-N membrane. The blot was hybridized at 60°C to a 570-bp *Pst* I fragment of the *Mgat-1* coding exon (12). The blot was reprobed with a 0.6-kb *Pst* I fragment of the human cardiac actin gene (27).

***Phaseolus vulgaris* Leukoagglutinin Binding to Embryos and Scanning Electron Microscopy.** Embryos free of visceral yolk sac were incubated with PBS containing 1 mM $MnCl_2$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 2% bovine serum albumin, fraction V (PBS/2%BSA; Sigma) at 4°C. After 30 min, the solution was replaced with PBS/2%BSA containing 20–40 μg of fluoresceinated L-PHA per ml (Vector Laboratories). After 30–60 min, each embryo was washed twice in 0.5 ml of PBS/2%BSA and examined by light and fluorescence microscopy with an Axiophot microscope. After lectin binding, some embryos were washed in PBS containing cations, dehydrated in a graded ethanol series, critical point dried, sputter coated with gold-palladium (Denton, Cherry Hill, NJ), and viewed at 10 kV in a JEOL JSM6400 scanning electron microscope.

Glycosyltransferase Assays. After lectin binding, embryos were washed separately at 4°C with 0.5 ml of PBS containing cations and suspended in 80 μl of 1.5% (wt/vol) Nonidet P-40 in sterile water. After 20 min on ice and gentle mixing, nuclei were removed by centrifugation for 3 min at 1000 × *g*. Glycerol (20 μl) was added to each extract before freezing at −70°C. GlcNAc-TI and β-N-acetylglucosaminylglycopeptide β-1,4-galactosyltransferase [β(1,4)Gal-T; UDPgalactose: *N*-acetyl-β-D-glucosaminylglycopeptide β-1,4-galactosyltransferase, EC 2.4.1.38] assays were performed with 10–15 μl of extract under optimized conditions and with the same substrates previously described (28).

RESULTS

Generation of ES Cells with a Disrupted *Mgat-1* Gene. A replacement targeting vector that would create a null mutation in the *Mgat-1* gene on chromosome 11 (11) was constructed (Fig. 1). No GlcNAc-TI activity was generated from this vector when it was transfected into Lec1 GlcNAc-TI$^-$ CHO cells (29). To obtain homologous recombinants, WW6 ES cells were electroporated with the linearized targeting vector (Fig. 1) and selected for resistance to G418 and ganciclovir. This positive/negative selection (30) resulted in a 2- to 3-fold enrichment of G418-resistant colonies. Two independent lines exhibiting the diagnostic 1.4-kb PCR fragment (Fig. 1) were obtained from 408 survivors. Genomic DNA from both WW6.15 and WW6.220 possessed the ≈3.8-kb *Bam*HI fragment predicted to arise from homologous recombination at the *Mgat-1* locus (Fig. 1). The neo probe also hybridized to a 2.7-kb *Bam*HI/*Kpn* I fragment specific to targeted cells (data not shown). As expected, WW6 cells and both recombinants exhibited the ≈10-kb *Bam*HI fragment from the *Mgat-1* gene (Fig. 1). Probes Y and Z also hybridized to an ≈5.7-kb *Bam*HI fragment (Fig. 1) that derives from the ≈1500 copies of the β-globin transgene in chromosome 13 of the WW6 genome (21, 31). The transgene was detected by all probes, presumably because of its content of short stretches of plasmid DNA. The *Bam*HI fragment of 5.7 kb is predicted from previous restriction analyses of this transgene (21, 31); *Hin*dIII digestion gave a predicted 4.9-kb fragment and *Bgl* II digestion gave a predicted 8.8-kb fragment from the transgene (data not shown).

Expression of the *Mgat-1* gene in recombinants was examined by Northern blot analysis (Fig. 2). Three heterozygous lines expressed ≈50% of the *Mgat-1* RNA found in WW6 cells. In addition, WW6.20 cells, selected in G418 to possess two disrupted *Mgat-1* alleles and shown to be homozygous *Mgat-1*$^{-/-}$ by Southern blot analyses, had essentially no *Mgat-1* RNA. The faint signal observed is probably due to feeder cell contamination since a transcript from the disrupted *Mgat-1* gene would be a different size. A null mutation was expected, as the reverse orientation of the neo cassette (Fig. 1) should prohibit transcriptional read-through into the coding region of the GlcNAc-TI gene.

Inactivation of the *Mgat-1* Locus Causes Embryonic Lethality. WW6.15 and WW6.220 were each injected into ≈70 blastocysts from C57BL/6 mice. Approximately 30–40 mice were born from each line and 25–35% of the progeny were chimeric based on coat color. Four males from WW6.15 and six from WW6.220 cells gave 100% transmission of the disrupted *Mgat-1* gene. The genotypes of the progeny of a mating between a male chimera and a C57BL/6 female are shown in Fig. 1. Both probes Y and Z detected the disrupted gene in the same 4 progeny and revealed the expected segregation of the β-globin transgene.

When mice heterozygous for the *Mgat-1* mutation were mated, no homozygous mutant progeny were obtained (Table 1). The ratio of homozygous wild-type to heterozygous progeny was ≈1:2.3. Although a comparatively small number of litters was examined, it seems that mice with a single copy of the *Mgat-1* gene were born at approximately the expected rate. In addition, the heterozygotes were indistinguishable morphologically from homozygous wild-type littermates and their fertility was excellent. In contrast, mice lacking a functional *Mgat-1* gene clearly died before birth.

As no *Mgat-1*$^{-/-}$ progeny were produced from heterozygous matings, embryos at different stages of development were examined. At 13.5 days postcoitus (dpc), 12 resorbed embryos were observed in four litters. Genotyping of the remaining embryos proved all of them to be +/+ or +/− at the *Mgat-1* locus (Table 1). Similar results were obtained at 12.5 dpc. However, at 11.5 dpc some of the smaller, abnormal looking embryos had two disrupted *Mgat-1* alleles. Therefore, in-depth characterization of progeny from 10.5, 9.5, and 8.5 dpc was performed.

Analyses of Mid-Gestation Embryos. Each embryo was examined for morphology by light microscopy and for binding of fluoresceinated L-PHA by fluorescence microscopy.

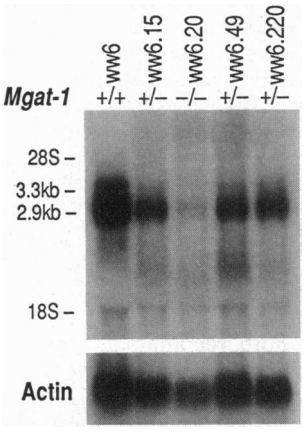

FIG. 2. Northern blot analysis of ES cell lines. All cell lines except WW6 were grown for one passage without a feeder layer and in the presence of leukemia inhibitory factor before extraction of total RNA. Blot was probed with a 575-bp *Pst* I fragment from the *Mgat-1* coding region, washed, and exposed for 5 days. Positions of the two *Mgat-1* mRNAs (11, 12) are indicated. Blot was subsequently rehybridized with a human cardiac actin probe.

Table 1. Genotype of progeny of heterozygous matings

Stage	No. of litters	Total progeny	Res.	*Mgat-1* genotype +/+	+/−	−/−
p1–p14	10	53	NA	16	37	0
E12.5–E13.5	5	41	17	7	17	0
E11.5	1	11	2	1	6	2*
E9.5–E10.5	10	102	5†	23	52	22

Res., resorbed; NA, not applicable; p, postnatal day; E, embryonic days.
*Another incomplete 11.5 dpc litter contained 2 *Mgat-1*$^{-/-}$ embryos.
†Appeared only in litters of 11 or more. Genotype from yolk sac DNA was *Mgat-1*$^{+/+}$ in one case.

This lectin is specific for N-linked carbohydrates and binds with high affinity to Gal residues on β-1,6-branched N-linked structures (32, 33). Lec1 CHO mutants that lack GlcNAc-TI activity are highly resistant to L-PHA and exhibit no cell surface binding of the lectin (34). After microscopy, the 10.5 and 9.5 dpc embryos were extracted with Nonidet P-40 for glycosyltransferase assays and DNA was prepared from pelleted nuclei.

From each 10.5 and 9.5 dpc litter analyzed, *Mgat-1*$^{-/-}$ embryos homozygous for either *Mgat-1* gene disruption were obtained in approximately the expected number (Table 1). These embryos had no detectable GlcNAc-TI activity (providing further evidence for a null mutation at the *Mgat-1* locus) but usually had good levels of another Golgi enzyme, β(1,4)Gal-T (Fig. 3). Littermates had similar specific activities for β(1,4)Gal-T. However, heterozygotes had about half the level of GlcNAc-TI activity, as expected if only one

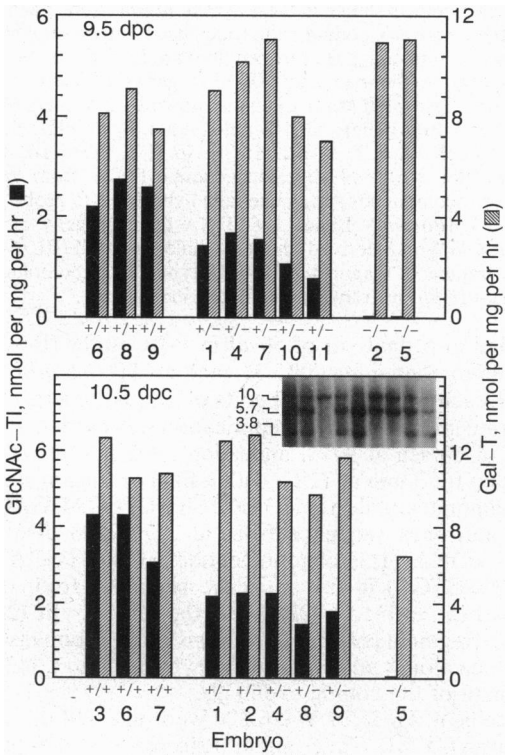

FIG. 3. GlcNAc-TI and β(1,4)Gal-T (Gal-T) glycosyltransferase activities. GlcNAc-TI assays were performed using the acceptor Man5GlcNAc2Asn and donor UDP-[6-^{3}H]GlcNAc; β(1,4)Gal-T assays used GlcNAc as acceptor and UDP-[6-^{3}H]galactose as donor. (*Upper*) Complete litter of female 26 at 9.5 dpc (embryo 3 was resorbed). (*Lower*) Complete litter of female 56 at 10.5 dpc. (*Inset*) Southern blot analysis of yolk sac DNA from 10.5 dpc progeny hybridized with probe Z (embryos 1–9 are numbered from left to right).

Mgat-1 allele were expressed. The ratio of β(1,4)Gal-T/GlcNAc-TI levels for 24 wild-type embryos was 3.2 ± 0.9; for 43 heterozygotes it was 6.3 ± 1.8—the ratio predicted based on *Mgat-1* gene dosage.

The *Mgat-1*$^{-/-}$ embryos from 9.5 and 10.5 dpc did not stain with L-PHA (Fig. 4 *Lower left*), showing that they do not express complex N-linked carbohydrates. In contrast, 24 wild-type embryos and 52 heterozygotes examined at these stages exhibited strong binding of fluorescein L-PHA (Fig. 4 *Lower right*). Embryos that did not bind L-PHA were also found in the expected ratio at 8.5 dpc. Therefore, no transferase substitutes for the missing GlcNAc-TI activity to produce complex N-linked carbohydrates at any of the stages examined.

Developmental Retardation of Embryos Lacking GlcNAc-TI. *Mgat-1*$^{-/-}$ embryos from 10.5 dpc were about one-quarter the size of littermates and deteriorating. Mutant embryos from 9.5 dpc, although small, were close in size to their smallest littermates. The most well-developed 9.5 dpc mutant embryo of 18 examined had ≈18 somites, an S-shaped heart, and a prominent bronchial arch I (Fig. 4 *Upper left*). The characteristic contours of the cephalic area were markedly diminished. In all *Mgat-1*$^{-/-}$ embryos, the cephalic area was generally amorphous and appeared disproportionately reduced in size. In addition, *Mgat-1*$^{-/-}$ embryos had enlarged tail areas, suggesting a potentially open neuropore.

In general, the *Mgat-1*$^{+/+}$ and *Mgat-1*$^{+/-}$ embryos of 9.5 dpc had 22–28 somites, whereas mutant embryos had 13–18 somites. Most mutant embryos had turned but none exhibited spiral torsion. In overall features, mutant embryos most resembled stage 14 (9 dpc) of normal embryos (35). However, *Mgat-1*$^{-/-}$ embryos were abnormally pale, suggesting anemia, and small blood spots were seen in the head and other regions.

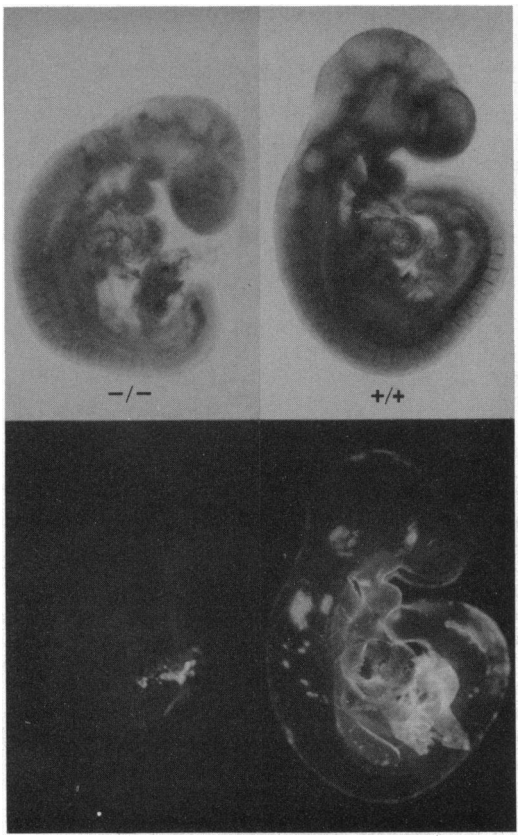

FIG. 4. Morphology and L-PHA binding of 9.5 dpc embryos. Embryos from 9.5 dpc were incubated with fluorescein L-PHA, washed, and examined by light (*Upper*) and fluorescence (*Lower*) microscopy. (*Left*) *Mgat-1*$^{-/-}$ embryo subsequently identified as embryo 2 in Fig. 3. Only a small piece of contaminating tissue bound L-PHA (*Lower left*). (*Right*) *Mgat-1*$^{+/+}$ littermate subsequently shown to be embryo 9 in Fig. 3. L-PHA staining was evident all over this embryo (*Lower right*). (×50.)

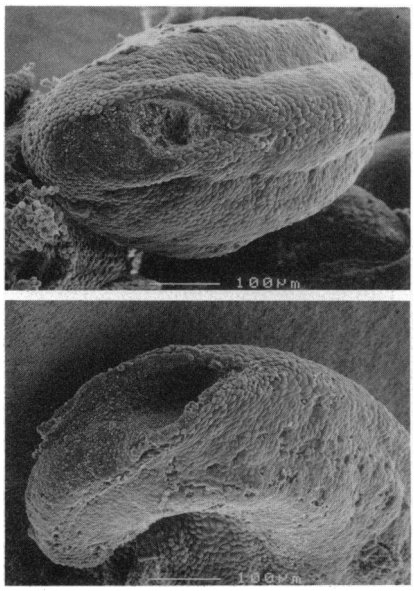

FIG. 5. Scanning electron microscopy of neuropores. Embryos were examined by scanning electron microscopy as described. Wild-type embryo (*Upper*) bound L-PHA and had normal morphology for 9.5 dpc. Mutant embryo (*Lower*) had a typical *Mgat-1*$^{-/-}$ morphology and did not bind L-PHA.

The features of four 9.5 dpc embryos identified as *Mgat-1*$^{-/-}$ by morphology and because they did not bind L-PHA and four littermates that had normal morphology and bound L-PHA were examined by scanning electron microscopy. All mutant embryos had wide open neuropores, whereas two of the normal littermates had a closed neuropore and the other two were almost closed (Fig. 5). One of the mutant embryos also had an open neural tube rostrally, indicating that it had developed to only about stage 13 (35). This was seen in some other *Mgat-1*$^{-/-}$ embryos by light microscopy but was not observed with littermates.

DISCUSSION

It has been known for more than a decade that mammals cannot survive without N-linked carbohydrates (36). However, it was difficult to predict how far a mouse would develop if missing only the complex and hybrid classes of N-linked carbohydrates. The results in this paper show unequivocally that these carbohydrates are essential for a mouse to develop beyond mid-gestation. No *Mgat-1*$^{-/-}$ embryos were found past 11.5 dpc, and those surviving to 9.5 or 10.5 dpc were small and underdeveloped. This phenotype is very similar to that described for mice with an inactivated c-*myc* (37), N-*myc* (38), or methyltransferase (39) gene and appears, from a preliminary report,† to be identical to that of an independently derived mouse mutated at the *Mgat-1* locus. In each of these mutants, substantial development of rudimentary organs and many tissues occurs in the absence of mutated activity. For the *myc* gene mutants, it is postulated that functional redundancy rescues the embryo to mid-gestation (37, 38). For the methyltransferase mutant, it is possible that large stores of maternal mRNA and partial

†Metzler, M., Gertz, A. & Marth, J. D. (1993) International Society of Development Biologists, p. 56, abstr. 188.

activity from the mutated methyltransferase alleles support early development (39).

It is expected that the *Mgat-1* gene is ubiquitously expressed in cells of the earliest embryo since glycoproteins with complex or hybrid N-linked structures have been obtained from four- to eight-cell embryos (40), although these could arise from maternal stores of *Mgat-1* gene products. If, however, it can be shown that, in contrast to normal embryos, *Mgat-1*$^{-/-}$ embryos never express complex or N-linked carbohydrates, it will prove that blastocyst and morula formation, compaction, and implantation proceed normally without these carbohydrates. This would eliminate hybrid or complex carbohydrates as mediators of the adhesion events in which sugars have been implicated during early mouse development (41).

A key challenge for the future is to determine the molecular basis for the demise of GlcNAc-TI-deficient embryos. Analyses of preimplantation embryos and early postimplantation embryos will be required to identify the earliest tissue abnormalities. However, important clues to the cause of death may also be obtained from analyses of the ability of *Mgat-1*$^{-/-}$ ES cell lines such as WW6.20 (Fig. 2) to differentiate. The β-globin transgene in the WW6 genome allows their tissue contribution to be readily monitored by *in situ* hybridization (21). It should therefore be possible to learn from chimeric embryos which tissues are initially affected by the lack of GlcNAc-TI. In addition, the ability of the *Mgat-1*$^{-/-}$ ES cells to form tumors in syngeneic hosts and to differentiate will provide insight into the differentiation potential of cells lacking GlcNAc-TI. The *Mgat-1*$^{-/-}$ cells can also be tested for their ability to contribute to T- and B-cell populations by attempting to form chimeras with recombination-activating gene (RAG)-deficient mice (42).

It seems from our limited studies that heterozygous mice with ≈50% GlcNAc-TI activity develop normally. Further insight into the biological requirement for GlcNAc-TI activity might be obtained from attempts to correct the deficiency in *Mgat-1*$^{-/-}$ embryos with a transgene expressing a weak GlcNAc-TI activity. Such an activity has been identified in the Lec1A CHO mutant (43). Partial restoration of GlcNAc-TI activity might allow further development of *Mgat-1*$^{-/-}$ embryos and reveal which tissues are initially aided by the ability to express some complex or hybrid N-linked carbohydrates.

The authors are very grateful to Elizabeth Robertson for cell lines and helpful discussions and to Ron DePinho for the actin probe, support, and advice. We also thank Jamey Marth for communicating results prior to publication; Jing Yang for the *Mgat-1 Sac* I subclone; Rudolf Jaenisch for neo and HSV-TK cassettes; Youxiang Wang and Yun Liu for mouse work; Subha Sundaram for glycosyltransferase assays; Frank Macaluso for help with microscopy; and T. Shantha Raju for preparing Fig. 3. This work was supported by Grant R37 30645 from the National Cancer Institute to P.S. and by Training Grant T32CA09060 from the National Institutes of Health. Support was also provided by Cancer Center Grant PO1 13330 from the National Cancer Institute.

1. Varki, A. (1993) *Glycobiology* **3,** 97–130.
2. Fukuda, M., Spooncer, E., Oates, J. E., Dell, A. & Klock, J. C. (1984) *J. Biol. Chem.* **259,** 10925–10935.
3. Maemura, K. & Fukuda, M. (1992) *J. Biol. Chem.* **267,** 24379–24386.
4. Fukushi, Y., Nudelman, E., Levery, S. B., Hakomori, S. & Rauvala, H. (1984) *J. Biol. Chem.* **259,** 10511–10517.
5. Bevilacqua, M. P. & Nelson, R. M. (1993) *J. Clin. Invest.* **91,** 379–387.
6. Lasky, L. A., Singer, M. S., Dowbenko, D., Imai, Y., Henzel, W. J., Grimley, C., Fennie, C., Gillett, N., Watson, S. R. & Rosen, S. D. (1992) *Cell* **69,** 927–938.
7. Moore, K. L., Stults, N. L., Diaz, S., Smith, D. F., Cummings, R. D., Varki, A. & McEver, R. P. (1992) *J. Cell Biol.* **118,** 445–456.
8. Kojima, N., Handa, K., Newman, W. & Hakomori, S. (1992) *Biochem. Biophys. Res. Commun.* **182,** 1288–1295.
9. Phillips, M. L., Nudelman, E., Gaeta, F. C. A., Perez, M., Singhal, A. K., Hakomori, S. & Paulson, J. C. (1990) *Science* **250,** 1130–1132.
10. Larsen, G. R., Sako, D., Ahern, T. J., Shaffer, M., Erban, J., Sajer, S. A., Gibson, R. M., Wagner, D. D., Furie, B. C. & Furie, B. (1992) *J. Biol. Chem.* **267,** 11104–11110.
11. Pownall, S., Kozak, C. A., Schappert, K., Sarkar, M., Hull, E., Schachter, H. & Marth, J. D. (1992) *Genomics* **12,** 699–704.
12. Kumar, R., Yang, Y., Eddy, R. L., Byers, M. G., Shows, T. B. & Stanley, P. (1992) *Glycobiology* **2,** 383–393.
13. Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54,** 631–664.
14. Stanley, P., Narasimhan, S., Siminovitch, L. & Schachter, H. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3323–3327.
15. Gottlieb, C., Baenziger, J. & Kornfeld, S. (1975) *J. Biol. Chem.* **250,** 3303–3309.
16. Stanley, P. (1989) *Mol. Cell. Biol.* **9,** 377–383.
17. Gleeson, P. A., Feeney, J. & Hughes, R. C. (1985) *Biochemistry* **24,** 493–503.
18. Hughes, R. C. & Feeney, J. (1986) *Eur. J. Biochem.* **158,** 227–237.
19. Fukuda, M. N., Dell, A. & Scartezzini, P. (1987) *J. Biol. Chem.* **262,** 7195–7206.
20. Fukuda, M. N., Masri, K. A., Dell, A., Luzzatto, L. & Moremen, K. W. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7443–7447.
21. Lo, C. W. (1986) *J. Cell Sci.* **81,** 143–162.
22. Rudnicki, M. A., Braun, T., Hinuma, S. & Jaenisch, R. (1992) *Cell* **71,** 383–390.
23. Mortensen, R. M., Conner, D. A., Chao, S., Geisterfer-Lowrance, A. A. T. & Seidman, J. G. (1992) *Mol. Cell. Biol.* **12,** 2391–2395.
24. Adra, C. N., Boer, P. H. & McBurney, M. W. (1987) *Gene* **60,** 65–74.
25. Robertson, E. J. (1987) *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach* (IRL, Oxford).
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
27. Rudnicki, M. A., Ruben, M. & McBurney, M. W. (1988) *Mol. Cell. Biol.* **8,** 406–417.
28. Chaney, W., Sundaram, S., Friedman, N. & Stanley, P. (1989) *J. Cell Biol.* **109,** 2089–2096.
29. Kumar, R. & Stanley, P. (1989) *Mol. Cell. Biol.* **9,** 5713–5717; erratum (1990) **10,** 3857.
30. Mansour, S. L., Thomas, K. R. & Capecchi, M. R. (1988) *Nature (London)* **336,** 348–352.
31. Tilghman, S. M., Tiemeier, D. C., Polsky, F., Edgell, M. H., Seidman, J. G., Leder, A., Enquist, L. W., Norman, B. & Leder, P. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4406–4410.
32. Hammarstrom, S., Hammarstrom, M.-L., Sundbland, G., Arnarp, J. & Lonngren, J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1611–1615.
33. Cummings, R. D. & Kornfeld, S. (1982) *J. Biol. Chem.* **257,** 11230–11234.
34. Stanley, P., Caillibot, V. & Siminovitch, L. (1975) *Somat. Cell Genet.* **1,** 3–26.
35. Kaufman, M. H. (1992) *The Atlas of Mouse Development* (Academic, San Diego).
36. Surani, M. A. H. (1979) *Cell* **18,** 217–227.
37. Davis, A., Wims, M., Spotts, G. D., Hann, S. R. & Bradley, A. (1993) *Genes Dev.* **7,** 671–682.
38. Charron, J., Malynn, B. A., Fisher, P., Stewart, V., Jeannotte, L., Goff, S. P., Robertson, E. J. & Alt, F. W. (1992) *Genes Dev.* **6,** 2248–2257.
39. Li, E., Bestor, T. H. & Jaenisch, R. (1992) *Cell* **69,** 915–926.
40. Iwakura, Y. & Nozaki, M. (1985) *Dev. Biol.* **112,** 135–144.
41. Kimber, S. J. (1990) *Int. Rev. Cytol.* **120,** 53–167.
42. Chen, J., Lansford, R., Steward, V., Young, F. & Alt, F. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4528–4532.
43. Chaney, W. & Stanley, P. (1986) *J. Biol. Chem.* **261,** 10551–10557.