We cloned a rat ABO homologue and established human A- and B-transferase transgenic rats. A DNA fragment corresponding to exon 7 of the human ABO gene was amplified from Wistar rat genomic DNA and sequenced. Using the amplified fragments as a probe for Southern blotting, multiple hybridized bands were found on both EcoRI- and BamHI-digested genomes of seven rat strains, which showed variations in the band numbers among the strains. Four cDNAs were cloned from a Wistar rat, three of which showed A-transferase activity and one of which showed B-transferase activity. These activities were dependent on the equivalent residues at 266 and 268 of human ABO transferase. Wild Wistar rats expressed A-antigen in salivary gland, intestine, and urinary bladder tissue, but B-antigen was not stained in any organs studied, whereas a transcript from the ABO homologue with B-transferase activity was ubiquitous. Human A-transferase and B-transferase were transferred into Wistar rats. A-transgenic rats expressed A-antigen in ectopic tissue of the brain plexus, type II lung epithelium, pancreas, and epidermis. B-antigen in the B-transgenic rat was expressed in the same organs as A-transgenic rats. These results may shed light on the function and evolution of the ABO gene in primates.
a single-locus gene. The antigen expression in wild rats and the effect of exogenous human genes will shed light on the function and the evolution of the ABO gene in primates.

MATERIALS AND METHODS

Probe Preparation and Southern Hybridization—Rat genomic DNA was extracted from the following rat strains: outbred Wistar, inbred DA/Slc, inbred LEW/Crlj, inbred PVG/Sea, inbred ACI/N Slc, inbred SD, and inbred BN+Crlj, which were maintained at Charles River Japan, Inc. (Yokohama, Japan). A DNA fragment corresponding to exon 7 of the human ABO gene was amplified from Wistar rat genomic DNA by PCR using a primer pair (sense, CAGACGACGACTCATGGTG, homologous to nucleotides 408 to 428; and antisense, GGGCCAGGGCCAGCACGTGCTGGTCCCA, complementary to nucleotides 970 to 996 of the human ABO cDNA). The PCR product was subcloned into pcR2.1 vector (Invitrogen, Carlsbad, CA) and sequenced using a Dye Terminator Cycle Sequencing kit (Beckman, Raritan, NJ). The rat genome DNA from the seven strains was digested by restriction enzymes, EcoRI or BamHI. The genome DNA fragments separated by agarose gel were blotted onto a membrane and hybridized with the 32P-labeled PCR products.

cDNA Cloning of Rat ABO Homologue—According to the results of the genome sequence, four clone-specific reverse primers were prepared for 5′-rapid amplification of a cDNA ends (RACE) procedure. Sense primers for 3′-RACE were then designed on sequences of the 5′-RACE. The RACE procedures were carried out using a Marathon™ cDNA-amplification kit (Clontech Laboratories, Inc., Palo Alto, CA) and rat genomic DNA. The total template RNAs were prepared from 15 Wistar rat organs: brain, submandibular gland, heart, lung, stomach, small intestine, large intestine, liver, pancreas, spleen, kidney, urinary bladder, testis, skin, and bone marrow. The PCR products were semi-quantified by gel electrophoresis followed by ethidium bromide staining.

Analysis of Enzymatic Activity of the ABO Homologue—Full-length cDNAs of rat ABO homologues were prepared by high fidelity RT-PCR with primers specific for 5′ and 3′ ends of each clone. Rat ABO homologues, human A-cDNA, and human B-cDNA were inserted into pcR3 (Invitrogen), and H-fructosyltransferase (FUT1) cDNA was inserted in pSS8b-bar vector (15). The enzymatic activities of four ABO homologues were assessed by the expression of A- or B-antigen on HeLa cells transfected by the cDNAs either with or without co-transfection of FUT1 cDNA. The cDNA expression was carried out by an electroporation procedure using GenePulser II (Bio-Rad). Twenty-four hours after transfection, HeLa cells were harvested and stained by anti-A, anti-B, or anti-H mouse monoclonal antibodies (Dako, Carpinteria, CA), and a second antibody of fluorescein isothiocyanate-labeled anti-mouse IgM goat serum (Cappel, Aurora, OH). The stained cells were analyzed by flow cytometry. The activity of the chicken β-galactosidase of the pCAGGS vector and injected into Wistar rat pronuclei. The microinjected fertilized eggs were transferred to pseudopregnant recipient rats. Transgenic founders were detected by PCR and RT-PCR of tail genomic DNA and total RNA, and stable lines were generated by breeding the founders.

Immunohistochemistry and Immunoblotting—Wild, A-transgenic, and B-transgenic rats were sacrificed at 3 and 5 months of age. The 15 organs and tissues were removed from the animals and embedded in compound. Ten-μm thick frozen sections were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer and placed in blocking buffer (phosphate-buffered saline, pH 7.4, 1% bovine serum albumin, 0.3% Triton X-100). The blocked sections were or were not reacted with anti-A, anti-B, or anti-H antibody. After washing, the sections were reacted with biotin-labeled anti-mouse goat antibody followed by Texas Red-labeled avidin staining. Counterstaining was performed with 4',6-diamidino-2-phenylindole (Sigma).

Small intestinal specimens from wild, A-transgenic, and B-transgenic rats were homogenized in 0.25 M sucrose solution and solubilized in SDS sample buffer. Ten micrograms per lane of solubilized small intestinal proteins were separated on a 5% polyacrylamide gel in denaturing buffer. After electrophoresis, proteins were electroblotted on a polyvinylidene difluoride membrane (Amersham Biosciences). The blot membrane was separated into three strips and the strips were stained individually with anti-A, anti-B, or anti-H antibody. Antibody-antigen complexes were visualized with peroxidase-conjugated goat anti-mouse IgM and West Pico chemiluminescent substrate (Pierce).

RESULTS

Southern Blot Analysis—To clone the rat ABO homologue gene, partial sequences were amplified from genome DNA, which corresponded to exon 7 of the human gene. Used primer sequences were chosen from the fragments highly conserved between human and mouse (17). The PCR product was subcloned into a plasmid and the individual clones were sequenced. Twenty sequenced clones were divided into four clones: rat-2.66 (AB081650), rat-2.6B (AB081651), and rat-3.47 (AB081652). The Southern blot membrane of the rat genome was probed by these fragments. The genome DNA digested by EcoRI showed variations in fragment number and size: four fragments in Wistar, DA/Slc, and ACI/N Slc; three fragments in LEW/Crlj and BN/Crlj; two fragments in PVG/Sea; and five fragments in SD rat (Fig. 1). DNA fragments that appeared in BamHI-digested genomes also showed polymorphisms among the strains: three fragments in Wistar, DA/Slc, and ACI/N Slc, and SD rats; and two fragments in LEW/Crlj, PVG/Sea, and BN/Crlj rats. These findings indicate that the rat ABO homologue consists of multicopy genes and the copy number possibly varies among strains.

cDNA Cloning of Rat ABO Homologue—The RACE procedure was used to obtain the full-length transcript sequences of the ABO homologues. Sixty-eight 5′-RACE and 48 3′-RACE clones were sequenced and realigned into four clones: rat-2.66 (GenBank™ accession number AB081649), rat-2.61 (AB081650), rat-2.6B (AB081651), and rat-3.47 (AB081652). These sequences were verified by a standard RT-PCR procedure with their 5′ and 3′ specific primers. The open reading
frames estimated were aligned with human and mouse transferases (Fig. 2A). The catalytic domain was conservative, but the regions following to the N-terminal transmembrane domain were variable. The rat-3.47 clone lost the fragment that corresponded to exon 4 of the human ABO gene and was also lost in mouse cDNA. The overlined amino acids in Fig. 2A show the four residues that are responsible for the substrate selection of human A- and B-transferase. Arrowheads indicate the boundaries of exons in human, suggesting the gap in rat-3.47 and mouse ABO corresponds to exon 4 of the human ABO gene. The rectangle indicates predicted transmembrane domains. B, full-length of the amino acid sequences of the rat, mouse, pig (AAC88440), crab-eating macaque (AAFO4724), rhesus monkey (AAD56306), and human were aligned and a phylogenetic tree was drawn. The neighbor joining method was used for Kimura’s distance. Mouse α-galactosyltransferase (MS5153) was used as an outgroup gene. The numbers on two interior branches are boot strap probabilities.

Based on the maximum matching analysis, a phylogenetic tree was drawn using the neighbor joining method (Fig. 2B). The four rat cDNAs were clustered in a branch next to the mouse ABO, suggesting that they are paralogous genes. "Enzymatic Activity of Rat ABO Homologues—Four rat cDNAs of the ABO homologues were inserted in a eukaryotic expression vector and their enzymatic activities were assayed by introducing the constructs into HeLa cells. The transfected HeLa cells were stained by anti-A, anti-B, and anti-H antibodies and subjected to flow cytometry (Table I). Three cDNA clones, rat-2.66, rat-2.61, and rat-2.6B, expressed A-antigen on HeLa cells when they were co-transfected with FUT1 cDNA, but clone rat-3.47 did not. Reciprocally, rat-3.47 expressed B-antigen but rat-2.66, rat-2.61, and rat-2.6B clones did not. Apparent expression of A- or B-antigens was not observed on the cells transfected with only ABO homologues (pSSR/H9251-bsr).
TABLE I
Flow cytometric analysis of HeLa cells transfected with expression vectors

| Rat ABO Genes of Paralogous Family |
|------------------------------------|
| Transfection | pSRα-FUT1 (+) | pSRα-FUT1 (+) |
|------------- | ------------- | ------------- |
| mock        | 0.5 (0.9 ± 1.4) | 0.5 (2.9 ± 1.3) |
| anti-H      | 2.8 (57.2 ± 13.4) | 73.7 (21.2 ± 26.1) |
| anti-A      | 3.0 (57.3 ± 13.0) | 0.4 (54.8 ± 13.5) |
| anti-B      | 2.6 (59.2 ± 12.5) | 66.4 (56.4 ± 15.3) |
| human-AAAA  | 1.5 (47.2 ± 12.0) | 0.4 (54.1 ± 14.7) |
| anti-H      | 1.1 (53.3 ± 10.8) | 36.7 (18.6 ± 24.6) |
| anti-A      | 1.3 (52.0 ± 11.1) | 66.4 (161.1 ± 20.0) |
| anti-B      | 2.0 (55.9 ± 10.8) | 0.3 (47.5 ± 12.3) |
| human-BBBB  | 1.2 (45.4 ± 12.0) | 0.1 (41.8 ± 11.1) |
| anti-H      | 2.3 (51.0 ± 11.9) | 44.6 (188.2 ± 41.9) |
| anti-A      | 1.7 (51.1 ± 10.9) | 0 (42.6 ± 10.8) |
| anti-B      | 2.7 (53.7 ± 11.1) | 58.1 (175.5 ± 26.6) |
| rat-2.66    | 0.5 (50.2 ± 12.7) | 0.5 (43.2 ± 11.7) |
| anti-H      | 2.6 (53.0 ± 11.8) | 93.2 (44.2 ± 15.4) |
| anti-A      | 5.3 (64.6 ± 17.4) | 64.6 (175.8 ± 17.4) |
| anti-B      | 3.3 (56.1 ± 12.0) | 0.6 (39.2 ± 12.9) |
| rat-2.61    | 1.7 (48.6 ± 11.8) | 0.2 (43.2 ± 12.3) |
| anti-H      | 1.7 (50.0 ± 11.3) | 3.2 (44.1 ± 14.0) |
| anti-A      | 5.3 (52.2 ± 11.1) | 88.2 (167.3 ± 17.1) |
| anti-B      | 2.7 (56.5 ± 11.7) | 0.3 (45.2 ± 12.5) |
| rat-2.6B    | 1.4 (48.2 ± 12.5) | 0.3 (43.2 ± 12.8) |
| anti-H      | 1.7 (50.9 ± 11.0) | 39.4 (177.3 ± 24.5) |
| anti-A      | 1.9 (51.5 ± 11.3) | 63.9 (162.1 ± 18.8) |
| anti-B      | 1.3 (54.1 ± 11.2) | 0.1 (45.4 ± 12.5) |
| rat-3.47    | 2.4 (50.8 ± 12.5) | 0.4 (41.6 ± 12.1) |
| anti-H      | 2.2 (51.8 ± 11.5) | 52.9 (170.1 ± 12.0) |
| anti-A      | 2.1 (52.8 ± 11.4) | 0.3 (49.2 ± 11.0) |
| anti-B      | 5.0 (58.3 ± 12.1) | 86.2 (177.3 ± 23.7) |

These results indicate that rat homologues also select H-antigen as an acceptor substrate and transfer GalNAc or Gal to it, depending on the residues responsible for substrate selection.

Expression Profile of Rat ABO Homologues—To discriminate the expression of the four rat ABO homologues, multiplex RT-PCR was used for the templates originating from the 15 organs. Simultaneous amplification in a tube and gel electrophoresis showed exclusive and relatively high level expression of rat-2.66 transcript in saliva glands, small intestine, and urinary bladder, and weak expression in the stomach and colon (Fig. 3). Weak bands from rat-2.61 and rat-2.6B appeared in the large intestine. Transcripts of rat-3.47 were ubiquitous.

Establishment of A- and B-Transferase Gene Transgenic Rats—Human A-transferase or B-transferase cDNA in pCAGGS vector was introduced in rat fertilized eggs by microinjection, and the founders were screened by PCR of tail DNA products. One microgram of total RNA was reverse transcribed into cDNA and amplified by PCR with specific primers for each cDNAs. PCR products were visualized on 2% agarose gel and stained with ethidium bromide. No enhanced expression of A-transferase or B-transferase activity has no effect on rat growth and development.

A- or B-Antigen Expression in Wild, A-Tg, and B-Tg Rats—To access the basal expression of A- and B-antigens in wild rats and determine the influence of overexpression of human A-transferase or B-transferase, systemic organs were stained by an immunohistochemical procedure. The staining profiles by anti-A or anti-B antibodies are listed in Fig. 4C and representative results are shown in Fig. 4B. Wild rats expressed A-antigens obviously on the acinus of salivary glands, mucosal crypts, intestinal mucus, and epithelium of the urinary bladder and weakly on stomach epithelium, which corresponded to the results of rat-2.66, rat-2.61, and rat-2.6B mRNA distribution. Enhanced expression of human A-transferase under the actin promoter resulted in the ectopic expression of A-antigen in the brain plexus, type II lung epithelium, the exocrine gland of the pancreas, and the epidermis, and enhanced expression in the epithelium of the stomach. Apparent staining by anti-B antibody was not observed in any wild and A-Tg rat organs studied, whereas weak expression of 3.47 mRNA was ubiquitous. Only B-Tg rats expressed B-antigen on the same organs as A-Tg rats, despite the fact that the staining was relatively weak compared with that in the A-Tg rats. The salivary glands, stomach, intestines, and bladder of B-Tg rats were still positive for anti-A antibody.

Western Blot Analysis of ABH Antigen Expression in the Intestine—To evaluate quantitatively the expression of ABH antigens in the intestine, the membranes blotted with small intestinal proteins of the three rats were stained by anti-A, anti-B, or anti-H antibody. It appeared that the bands with molecular weights around 216,000 and 78,000 were the non-specific immune complexes or intrinsic peroxidase activity. Monoclonal antibody against A-antigen showed equivalent smear bands in all three rats. Antibody against B-antigen stained only B-Tg rat intestinal proteins. Anti-H antibody stained all rats weakly, but the smear band in wild rat was most prominent, which might be the result of masking of residual H-antigen by the overexpression of human A-transferase or human B-transferase (Fig. 5).

DISCUSSION
We cloned four paralogous gene family members equivalent to the ABO glycosyltransferase gene from a Wistar rat. Three of the genes expressed A-antigen and one expressed B-antigen on...
HeLa cells with the help of FUT1. The amino acid residues responsible for the selection of the nucleotide sugar donor in humans (L266M and G268A) were Ala and Gly in rat-2.66, rat-2.61, and rat-2.6B clones and resembled human A-transferase, and Met and Ala in rat-3.47 clones were identical to human B-transferase. These results showed that the corresponding residues of human 266 and 268 in rat clones are involved in the nucleotide sugar donor selection, UDP-GalNAc or UDP-Gal. B-antigen was undetectable in any organs studied from wild Wistar rats, whereas rat-3.47 mRNA for B-transferase activity was expressed ubiquitously in rat organs. Enforced expression of human B-transferase, however, resulted in the expression of B-antigen, although the level was much less than that of A-antigen. Therefore, the low transcript level of rat B-transferase may be the main reason for the deletion of B-antigen in wild Wistar rats. Another possible reason is competition for the substrate of UDP-galactose with 1,3-galactosyltransferase, which was discussed in a mouse homologue with cis-AB activity (17).

The organs of wild, A-transgenic, or B-transgenic rats were stained with anti-A or anti-B antibodies, then with biotin-labeled anti-mouse goat antibody, and finally with Texas Red-labeled avidin (red). Nuclei were stained by 4',6-diamidino-2-phenylindole (blue). A, A- and B-antigen expression in the tails. A-antigens were detected on the cell surface of the epidermis, hair follicle epithelium, and sweat glands of A-transferase transgenic rats but not in B-transferase transgenic rats. B-antigen was detected in the same tissue only in B-transferase transgenic rats. B-antigen was detected in the same tissue only in B-transferase transgenic rats. B-antigen was detected in the same tissue only in B-transferase transgenic rats. B-antigen was detected in the same tissue only in B-transferase transgenic rats.

![Fig. 4. Immunostaining of A- or B-antigen in wild, A-transgenic, or B-transgenic rat organs. The organs of wild, A-transgenic, or B-transgenic rats were stained with anti-A or anti-B antibodies, then with biotin-labeled anti-mouse goat antibody, and finally with Texas Red-labeled avidin (red). Nuclei were stained by 4',6-diamidino-2-phenylindole (blue). A, A- and B-antigen expression in the tails. A-antigens were detected on the cell surface of the epidermis, hair follicle epithelium, and sweat glands of A-transferase transgenic rats but not in B-transferase transgenic rats. B, A-antigen and B-antigen expression in organs. 1, small intestine of A-transgenic rat stained with anti-A antibody. 2, small intestine of B-transgenic rat stained with anti-A antibody. 3, small intestine of A-transgenic rat stained with anti-A antibody. 4, small intestine of B-transgenic rat stained with anti-B antibody. 5, lung of A-transgenic rat stained with anti-A antibody. 6, brain of A-transgenic rat stained with anti-B antibody. 7, pancreas of A-transgenic rat stained with anti-A antibody. 8, salivary gland of wild rat stained with anti-A antibody. 9, urinary bladder of wild rat stained with anti-A antibody. 10, large intestine of wild rat stained with anti-A antibody. C, list of immunostaining results of systemic organs of wild, A-transgenic, and B-transgenic rats. Results are displayed as positive (+) or negative (−) staining against anti-A or anti-B monoclonal antibody.]

![Fig. 5. Western blot analysis of rat small intestine. Ten micrograms per lane of solubilized small intestinal proteins from wild, A-transgenic, or B-transgenic rats were separated on 5% SDS-PAGE gel. After electrophoresis, proteins were electroblotted onto a membrane. The blot membrane was separated into three strips and the strips were stained by anti-A, anti-B, or anti-H antibody.](http://www.jbc.org/)

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Bouhours et al. (9) reported that rat large intestinal glycosphingolipids showed genetic polymorphisms, in which some rat strains lacked A-antigen, whereas all strains expressed B-antigen active glycosphingolipid. By breeding and backcrossing of the strains, those investigators also revealed that the polymorphism was not the result of allelic genes but was controlled by closely linked multigene. During the preparation of this paper, Olson et al. (19) reported on two rat A-transferases they cloned as a pair of alleles (AF469945 and AF469946). The nucleotide and amino acid sequences of AF469946 are identical to those of rat-2.61, and AF469945 is 95% homologous to rat-2.66 and rat-2.61 clones. However, our four cDNA clones were identified from one animal and each mRNA expression pattern is distinctive from the others. Furthermore, multiple bands in a Southern blotting study supported a conclusion that four Wistar rat genes equivalent to the human histo-blood group ABO gene are not the allelic genes but members of the paralogue gene family.

The predicted amino acid sequences of rat-2.66, rat-2.61, rat-2.6B, and rat-3.47 showed 97 to 83% homology among them. The neighbor joining method revealed that the rat orthologue members form one cluster, indicating they evolved from an ancestral gene. However, multiple alignment of the four predicted peptides showed sharing of amino acid residues among them, i.e. between rat-2.66 and rat-2.61 and between rat-2.66 and rat-2.6B. This indicated that the amino acid differences among the genes did not simply result from base substitutions but recombination by repeated gene shuffling among them. It has been postulated that tandem copy paralogous genes evolve through interlocus nucleotide shuffling. For example, RHID and RHCE genes, encoding the rhesus blood group system, donate or accept gene fragments from each other and develop highly polymorphic antigen systems (20, 21). Rat ABO homologues might have evolved in this manner, and some strains lack A-transferase activity in the large intestine. The multicopy status of the ABO homologue appears not to be restricted to rats. Multiple fragments in the Southern blotting study probed by the human A-transferase gene were found in dogs, cats, and rabbits in addition to rats (22). Expression of both A- and B-antigens in the intestinal mucosa of rabbits has also been reported (23).

Saitou and Yamamoto (24) argued that the seven nucleotide polymorphisms between A and B alleles in human are more excessive than expected as simple base substitutions and that positive selection pressure affected the evolution of higher pri-mate ABO genes. However, if the mammalian ABO ancestral genes consisted of multicopy genes with A- and B-transferase activity like rats, they must donate or accept gene fragments from each other and may produce a locus composed of A and B alleles. The multicopy genes may possibly gather and bundle into a single gene through unequal crossover. Southern blotting studies in seven rat strains suggest deletion or acquisition of some loci. This estimation must be resolved by further genome projects in rats and other mammals.

A-Tg rats expressed A-antigen in the brain plexus, lung type II epithelium, pancreas acinus, and skin in addition to digestive mucosa, salivary gland, and bladder, in which A-antigen was expressed in wild rats also. B-antigen in B-Tg rats was detected in the same tissue as A-antigen in A-Tg rats. These results indicate that the organs that ectopically expressed A-antigen provided the substrates for the transferase, H-antigen, but lacked the endogenous ABO homologues and resulted in negative staining against anti-A antibody. H-antigen in rodents is restricted to epithelial organs, whereas they also have two α1,2-fucosyltransferase genes like human (25, 26). The rodent orthologue of human FUT1 lacks the promoter that transactivates the gene in the erythroid of hominoids, so that rodents do not express H-antigen in the organs of mesothelial origin (27, 28). Ubiquitous and overexpression analysis of A-transferase and B-transferase clearly showed the absence of H-antigen in the endothelium, brain plexus, lung, and pancreas. Further transgenesis of the human FUT1 gene will mimic ABH expression in human organs and will be useful for the simulation of the ABO-mismatched vascular graft.

Holmes et al. (12) reported that precancerous liver and hepatoma in Fischer 344 rats induced by the chemical carcinogen N2-acetylaminofluorene expressed B-antigen as the α-galactosyl-α-fucosyl-GM3 form. Precancerous liver and hepatome were induced to express α-fucosyltransferase (29). The synthesized α-fucosyl-GM3 was the substrate for normally expressing α-galactosyltransferase and was converted into B-determi-nants. The ABO homologue that liver is expressing is the rat-3.47 gene with B-transferase activity. Then, the normally existing α-galactosyltransferase is estimated to have been the product of the rat-3.47 gene. In human colorectal, lung, and bladder cancer, the deletion of A- or B-antigen in cancer cells associate with poor prognosis of the cancer patients (7). In 25% of primary bladder tumors, loss of heterozygosity of the ABH locus was observed (30). Rat chemical-induced colorectal cancer also changed the ABH antigen expression (13). The ABH gene expression was down-regulated in A-negative human colorectal cancer cells by the methylation of the promoter and the reduced activity of the enhancer element of the gene (31, 32). The presented cDNA data should contribute for investigation in alteration of the rat ABO homologue expression in the chemical oncogenesis. Furthermore, the established Tg rats express A- or B-transferase independently from the native genes and will be useful to reveal whether the ABH antigen affect directly the cancer virulence.

ABH antigen expression in human and rat fetuses is under strict control by stage of development and it is organ-specific (6, 33), whereas its exact meaning for fetal development is still under investigation. ABH antigen was found in early embryos (week 5) in the cardiovascular endothelium, the epithelial cells of all organ rudiments, and the erythropoietic cells in blood islands (6). After recession of the epithelial cell wall antigens at the end of the first trimester of pregnancy, ABH antigen expression increases as the respective organs mature, e.g. mucous secretion in digestive organs. A-antigen and B-antigen expression in transgenic rats should be controlled by FUT1 or FUT2 gene expression, which might permit the usual embryogenesis of the transgenic rats. Immunohistochemical follow-up of A-antigen expression in wild and transgenic rat fetuses may provide further information on the function of the ABH antigen in embryogenesis.

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