**Pax6** Controls the Expression of Lewis x Epitope in the Embryonic Forebrain by Regulating α,3-Fucosyltransferase IX Expression*

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**Pax6** is a transcription factor involved in brain patterning and neurogenesis. Expression of **Pax6** is specifically observed in the developing cerebral cortex, where Lewis x epitope that is thought to play important roles in cell interactions is colocalized. Here we examined whether **Pax6** regulates localization of Lewis x using **Pax6** mutant rat embryos. The Lewis x epitope disappeared in the **Pax6** mutant cortex, and activity of α,3-fucosyltransferase, which catalyzed the last step of Lewis x biosynthesis, drastically decreased in the mutant cortex as compared with the wild type. Furthermore, expression of a fucosyltransferase gene, FucT-IX, specifically decreased in the mutant, while no change was seen for expression of another fucosyltransferase gene, FucT-IV. These results strongly suggest that **Pax6** controls Lewis x expression in the embryonic brain by regulating FucT-IX gene expression.

The development of the central nervous system requires the specification of distinct regions, which presumably results from spatiotemporally restricted expression of various regulatory genes, including **Pax6**. **Pax6** is expressed in several discrete domains of the developing central nervous system (Refs. 1 and 2, see also review by Osumi (3)). **Pax6** protein is a transcription factor containing two DNA-binding motifs (a paired domain and a paired-type homeodomain) (4). Molecular and morphological analyses of the **Pax6** mutant mice and rats suggest that **Pax6** is crucial for the normal development of the forebrain (5–14). **Pax6** is expressed in the cortex but not in the striatum of the telencephalon (1, 2). **Pax6** mutant fails to establish the boundary between the cortical and striatal regions (6, 8, 14–18). It has been proposed that Ca2+-dependent selective adhesion of the cortical cells segregating from the striatal cells contributes to the formation and maintenance of boundaries between these telencephalic regions (19). In **Pax6** mutant, this selective adhesion of cortical cells is lost (16) and neural cell migration from the striatum into the cortex is strongly enhanced (20).

It has been reported that the cortex of the rat telencephalon at embryonic days 12 to 15 (E12–15)1 distinctively expresses Lewis x epitope (19, 21, 22). The Lewis x epitope, which is also known as CD15 and SSEA-1 (stage-specific embryonic antigen-1), has been identified as a glycan epitope with a Galβ1–4(Fucα1–3)GlcNAcβ1- structure (23–25). It has been proposed that Lewis x glycans interact with each other in a Ca2+-dependent manner (26, 27). The expression of the Lewis x epitope is highly regulated during embryogenesis. The epitope appears in the mouse embryo at the morula stage and decreases rapidly after compaction (28), and it has been demonstrated that the multivalent Lewis x epitope induces decompaction of the embryo (29). Several immunohistochemical and biochemical studies have demonstrated that the expression of Lewis x epitope is spatiotemporally regulated in the developing central nervous system (21, 30–34). However, the mechanism by which Lewis x epitope expression is regulated during embryogenesis remains unknown.

α,1,3-Fucosyltransferase (α3FucT) catalyzes the final step of the biosynthesis of the Lewis x epitope. A series of mammalian α3FucT has been cloned to date. In human, six members of the α3FucT gene family, FucT-III (35), -IV (36–38), -V (39), -VI (40, 41), -VII (42, 43), and -IX (44), have been identified and a subfamily, consisting of FucT-III, -V, and -VI, forms a gene cluster (45). In mouse, a homologue of FucT-III/V/VI has been reported to be a pseudogene (46), while FucT-IV, FucT-VII, and FucT-IX homologues are functional genes (46–49). Each member of the α3FucT family differs in its ability to synthesize Lewis x and/or sialyl Lewis x epitopes. While FucT-III, -V, and -VI synthesize both epitopes (39, 40), FucT-VII synthesizes only sialyl Lewis x (42, 43, 48), and FucT-IV and -IX synthesize Lewis x but little or no sialyl Lewis x (37, 44, 49).

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1 The abbreviations used are: E, embryonic day; α3FucT, α,1,3-fucosyltransferase; HPTLC, high performance thin-layer chromatography; PA-glycan, glycan labeled with 2-aminopyridine; Cer, ceramide; VZ, ventricular zone; CSPG, chondroitin sulfate proteoglycan; RPTP-ζ/β, receptor-type protein-tyrosine phosphatase-ζ/β; β4GalT, β1,4-galactosyltransferase; LAD II, leukocyte adhesion deficiency type II.
In this study, we examined the relationship between Pax6 and the expression of Lewis x epitope in the developing nervous system. Our preliminary observations led us to hypothesize that Pax6 regulates the expression of Lewis x epitope by controlling α3FucT activity. To test this hypothesis, we investigated the expression of Lewis x epitope and α3FucT activity in the rat E13.5 telencephalon of the wild type and the Pax6 mutant. By investigating the expression of FucT-IV and FucT-IX in E13.5 telencephalon, we demonstrated that the expression of Lewis x epitope is through regulating the gene expression of FucT-IX by Pax6.

EXPERIMENTAL PROCEDURES

Animals—Rat Small eye (r Sey2) strain was used as Pax6 mutant (50), and Sprague-Dawley strain was used as the wild type. Homozygous embryos were obtained from intercrosses of heterozygous rSey2 rats. The day when the vaginal plug was found was designated as embryonic day 0 (E0). Homozygous embryos could easily be distinguished from those of the wild type from their external features; the former lacked the eye and nose primordia.

Immunostaining and in situ Hybridization—For histological analyses, immunostaining and in situ hybridization were performed on frozen sections of Pax6 mutant and wild type rat embryos according to the methods described previously (50–52). Anti-Pax6 rabbit antibody (53) was used at 1:500, anti-Lewis x monoclonal antibody (clone 73–30; Seikagaku) at 1:500, and Leu7 antibody (the same as HNK-1; Becton Dickinson) was at 1:25. RNA probes for rat Pax6 (50), FucT-IV, and FucT-IX (as described below) were generated using T7 or T3 RNA polymerase.

Immunoprecipitation and Immunoblotting—Proteins were extracted from the telencephalon dissected from three embryos at E13.5. The specimens were homogenized by sonication for 15 s in distilled water. Neutral glycolipids were isolated from the homogenate and the resulting supernatant, 5°C sucrose containing 1 mM EDTA by sonication for 15 s, followed by centrifugation at 10,000 × g for 3 min. The protein concentrations were determined by the Bio-Rad protein assay kit.

FucT-IV fragment thus obtained was cloned into pGEM-T Easy vector (Promega) and the subcloned fragments were subcloned into the pCDM8 vector. COS-7 cells (1 × 10⁴ cells) were transfected with one of the expression plasmids using LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. After 3 days, the cells were harvested, washed with phosphate-buffered saline, homogenized in 100 μl of 0.3% Triton X-100, and used as the enzyme source for the αL3-fucosyltransferase assay.

Quantification of mRNA by Real-time Detection-PCR—Real-time detection-PCR was performed in a set of PCR using a TaqMan gene expression assay complementary to the sequence located to nonconverged regions between FucT-IV and Pax6. Oligonucleotides were used as follows: FucT-IV forward, 5'-GGTTGAGAACAGACAGCAG-3'; FucT-IX forward, 5'-CAAGCTTCCCTGCTGG-3'; FucT-IV reverse, 5'-GCTAGACAGCAGCACAG-GTG-3'; FucT-IX reverse, 5'-GCGAGGCATGTTCCTCCT-3'; and the TaqMan probe, 5'-TCCACAGCCATGTTCCTCCT-3'. The TaqMan probe was attached with the reporter dye FAM to the 5'-end and the quencher dye TAMRA to the 3'-end. Standard RNAs were synthesized using FucT-IV or FucT-IX cloned into Bluescript II as a template and T7 RNA polymerase and MEGAscript in vitro transcription kits (Ambion). Synthetic RNA was treated with DNase I and purified using an RNasy column (Qiagen). The possibility of contamination of the DNA template in the purified RNA was eliminated by PCR without reverse transcription. Synthetic RNA copy numbers were calculated from the quantity and molecular weight of RNA according to conventional methods. Real-time detection-PCR was performed using a GeneAmp Ez rTh RNA PCR kit and an ABI PRISM 7700 Sequence Detector (PE Biosystems) as described previously (58), except that the reaction mixture contained 50 μl forward primer and 300 μl reverse primer.

RESULTS

Expression of Lewis x Epitope on Telencephalic Cortex Disappears in the Pax6 Mutant—Whole-mount in situ staining of the dissected brain revealed Pax6 mRNA expression in the E13.5 rat dorsal telencephalon (Fig. 1F), as was observed in mouse embryos (1, 2). Immunohistochemistry using anti-Pax6 antibody showed intense staining in the ventricles and the rostral thalamus of the wild type (Fig. 1C). We examined the expression of Lewis x epitope in the telencephalon of both wild type and Pax6 mutant embryos. The ventricular and telencephalic cortex was stained with anti-Lewis x antibody in wild type E13.5 embryo (Fig. 1A), as was reported in the E12–15 rat forebrain (19, 21, 22). The expression of Lewis x epitope was not detected in the dorsal thalamus where Pax6 staining was positive, but was detected...
in the lateral and medial ganglionic eminences where Pax6 staining was negative (Fig. 1, A and C). Thus the expression patterns of Pax6 and Lewis x epitope partially overlapped in the wild type telencephalon. In the homozygous embryo, however, little staining with anti-Lewis x antibody was observed in the telencephalic cortex (Fig. 1D). In contrast with Lewis x, no differences in the expression of HNK-1 epitope in the outer layer of the cortex and the broad area of the basal ganglia (51) were observed between the wild type and homozygous embryos (Fig. 1, B and E). These results suggest that the expression of Lewis x epitope in the telencephalic cortex is regulated by Pax6.

Lewis x Epitope on Proteoglycan Disappears in the Pax6 Mutant—Lewis x epitope has a Galβ1-4(Fucα1–3)GlcNAcβ1-structure at the terminus of the glycan and is present on some glycoconjugates (glycoproteins, glycolipids, proteoglycans). The following two mechanisms may account for our immunohistochemical results of Lewis x (Fig. 1, A and D): 1) the carrier glycoconjugate itself disappears in the homozygote or 2) the glycan on the existing carrier in the homozygote differs from the Lewis x-positive one in the wild type. To determine the molecular basis of the regulation of Lewis x expression by Pax6, we searched for Lewis x glycan-bearing materials that exist in the wild type tissue but not in the homozygous brain.

Proteinaceous materials from E13.5 telencephalon were immunoprecipitated with anti-Lewis x antibody (clone 73-30) and subjected to immunoblotting analysis using anti-Lewis x antibody (clone 73-30) and subjected to immunoblotting analysis using anti-Lewis x antibody (clone 73-30). A positive band was observed at the position corresponding to ~950 kDa in the immunoprecipitate from the wild type but not from the homozygote (Fig. 2A). The mobility of the positive band was increased to the position corresponding to ~1,400 kDa upon treatment with chondroitinase ABC, which catalyzes the removal of chondroitin sulfate side chains from proteoglycans (Fig. 2B, lane 2). The positive band disappeared after treatment with endo-β-galactosidase which hydrolyzes β-galactoside linkage in poly-N-acetyllactosamine (Fig. 2B, lane 3) or peptidglycanase A, which acts on N-linked glycan (Fig. 2B, lane 4). These results indicate that the Lewis x-positive protein is a chondroitin sulfate proteoglycan (CSPG) and that the Lewis x epitope is present on a poly-N-acetyllactosamine structure on the N-linked glycan on the CSPG. While these features are common to phosphacan/receptor-type protein-tyrosine phosphatase-γβ (RPTP-γβ) (22, 59, 60), it has been suggested on the basis of immunoblotting and immunohistochemistry using anti-phosphacan antibodies that Lewis x-CSPG is probably distinct from phosphacan/RPTP-γβ. The CSPG has yet to be identified.

Neutral glycolipids extracted from the E13.5 telencephalon were subjected to HPTLC and stained with anti-Lewis x antibody (clone 73-30). Lewis x-positive bands were observed in material obtained from both the homozygote and the wild type (Fig. 2C). The simplest form of Lewis x-glycolipid was not detected. It is likely that Lewis x-glycolipid in the E13.5 telencephalon have a more complex structure, with the major one probably being Galβ1-4(Fucα1–3)GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glcβ1–1Cer as described for rat E15 cerebral cortex by Chou et al. (34).

a1,3-Fucosyltransferase Activity in Telencephalic Cortex Decreases in the Pax6 Mutant—As described above, Lewis x-bearing CSPG was detected in the wild type but not in the homozygote, whereas Lewis x-glycolipid was detected in both the homozygote and the wild type. The most reasonable explanation of the disappearance of Lewis x epitope in our immunohistochemical analysis (Fig. 1B) is that the expression of the core protein of the CSPG in the homozygote is down-regulated. Another possibility is the abnormal expression of a type of glycosyltransferase that is specific to, or preferential for, glycan on the CSPG rather than glycolipid.

Since Lewis x glycan is synthesized from the N-acetyllactosamine (Galβ1–4GlcNAc) structure at the terminus of the glycan on glycoconjugate to which fucose is added as the final step by a1,3-fucosyltransferase (α3FucT), we assayed α3FucT activity in the E13.5 telencephalon. As acceptor substrates, we used a PA-glycan and a glycolipid, both of which have an N-acetyllactosamine structure at the terminus. The telencephalon
analyzed the activity of FucT-IV and FucT-IX decreased to 16 and 33%.

ture, a precursor of Lewis x glycan. The reaction product was detected in E13.5 cortex (Fig. 3).

FucT-IV showed little synthesis of sialyl Lewis x glycan in contrast with FucT-IX in the E13.5 telencephalon. As the telencephalic cortex, which expresses Lewis x epitope (Fig. 1A), was used as an enzyme source. Data are expressed as specific activity (percent) relative to the value of the wild type telencephalic cortex. Error bars indicate the S.E. of the mean.

One of the simplest hypotheses that may account for the expression of FucT-IV and FucT-IX in the rat embryonic brain, we first cloned rat FucT-IV and FucT-IX cDNAs using a PCR-based approach. The nucleotide sequences obtained in this study were registered in DDBJ/GenBank (accession numbers: AB049938 (FucT-IV) and AB049819 (FucT-IX)).

To determine the enzymatic properties of the two cloned rat FucT-IVs, we analyzed the α3FucT activity of COS-7 cells transfected with FucT-IV or FucT-IX. Both homogenates of these transfecants showed α3FucT activities toward both PA-glycan and glycolipid (Table I, see also Figs. 2C and 3C). Cells transfected with mock vector showed little activity (Table I and Fig. 3C). These results indicate that both rat FucT-IV and FucT-IX synthesize Lewis x epitope. Interestingly, the specific activity of FucT-IX-transfectant toward PA-glycan was 4-fold that of FucT-IV-transfectant, while their specific activities toward glycolipid were very similar (Table I). This observation suggests that FucT-IX may act on soluble acceptor substrate more efficiently than FucT-IV.

Expression of FucT-IX Decreases in the Pax6 Mutant—As mentioned above, the expression of FucT-IV and FucT-IX may be regulated by Pax6. Thus, using conventional reverse transcription-PCR, we examined whether FucT-IV and FucT-IX are expressed in the E13.5 rat telencephalon. Both FucT-IV and FucT-IX products were amplified using telencephalic RNA of both the homozygote and the wild type (data not shown). We then investigated the localization and quantification of their transcripts in the telencephalon. In situ hybridization analysis showed a striking contrast between the expression patterns of FucT-IV and FucT-IX in the E13.5 telencephalon (Fig. 5). Weak FucT-IV expression was detected in the broad area of the telencephalon in either the wild type or the homozygote (Fig. 5, D and H). On the other hand, FucT-IX expression was localized in the VZ of the cortex and dorsal half of the medial wall in the wild type telencephalon (Fig. 5C), where FucT-IX expression was co-localized with Pax6 (Fig. 5A; see also Fig. 1C). In the dorsal thalamus where Pax6 was expressed, however, little FucT-IX expression was detected. Interestingly, FucT-IX expression was strongly reduced in the telencephalic cortex in the homozygote (Fig. 5G).

To quantify the transcripts of FucT-IX and FucT-IV in the telencephalic cortex, we used a real-time detection-PCR system. The sensitivity and linearity of the assay were examined using synthetic FucT-IV and FucT-IV RNA (data not shown). The quantities of FucT-IX, FucT-IV, and GAPDH (internal standard) transcripts in the telencephalic cortex were determined using three or four each of wild type and homozygous embryos. The relative quantities of FucT-IX and FucT-IV tran-
TABLE I
\(\alpha_1,3\)-Fucosyltransferase activities of rat FucT-IV and FucT-IX expressed in COS-7 cells

| Specific activity, acceptor substrate | PA-glycan | Glycolipid |
|--------------------------------------|-----------|-----------|
| **FucT-IV**                          | 2.12 \times 10^4 (100) | 11.6 (100) |
| **FucT-IX**                          | 7.98 \times 10^4 (376) | 12.2 (105) |
| Mock vector                          | 0.04 \times 10^3 (2) | 0.4 (3) |

| pmoi/h/mg protein |
|-------------------|
| PA-glycan         |
| Glycolipid        |

| Specific activity, acceptor substrate | PA-glycan | Glycolipid |
|--------------------------------------|-----------|-----------|
| **FucT-IV**                          | 2.12 \times 10^4 (100) | 11.6 (100) |
| **FucT-IX**                          | 7.98 \times 10^4 (376) | 12.2 (105) |
| Mock vector                          | 0.04 \times 10^3 (2) | 0.4 (3) |

scripts were expressed as copy numbers relative to that of GAPDH transcript, which was taken as \(1.0 \times 10^5\) (Fig. 6). The amount of \(FucT-IX\) transcript in the homozygote was about three times less than that of the wild type, while little difference was noted for the values of \(FucT-IV\). This result is consistent with that obtained by \textit{in situ} hybridization, and both results strongly suggest that \textit{Pax6} regulates the gene expression of \(FucT-IX\) in the rat embryonic brain.

**DISCUSSION**

In this study, we demonstrated that the Lewis x epitope, which is localized in E13.5 rat telencephalic cortex, disappears in the \textit{Pax6} homozygous mutant. Enzymatic activity of \(\alpha_3\)FucT, which catalyzes the final step of Lewis x biosynthesis, decreased in the homozygote, compared with the wild type. The telencephalic cortex that expressed \textit{Pax6} co-expressed \(FucT-IX\), a member of the \(\alpha_3\)FucT family. Moreover, the \(FucT-IX\) expression also decreased in the \textit{Pax6} mutant. These results suggest that a functional \textit{Pax6} regulates, either directly or indirectly, the gene expression of \(FucT-IX\) and, as a consequence, the localization of Lewis x epitope. The transcripts of \(FucT-IX\), however, were detected in the homozygote by reverse transcription-PCR as one third of the wild type (Fig. 6A). On the other hand, \(FucT-IX\) was not detected \textit{in situ} hybridization in the wild type dorsal thalamus where \textit{Pax6} is expressed (data not shown). It seems likely that distinct transcription factor(s) may regulate the expression of \(FucT-IX\) gene at a basal level and that \textit{Pax6} can act synergically to promote \(FucT-IX\) expression to a functional level. It has been reported that a \textit{Pax6} protein binds to the promoter region of \(L1\) (62, 63), \(\alpha\)-crystallin (64), or \(N\)-CAM gene (65). The recognition motifs, which are necessary for binding to \textit{Pax6} protein, have been reported (63–66). To elucidate the regulatory mechanism of the \(FucT-IX\) gene expression, we are now analyzing the 5ʹ-flanking region of the \(FucT-IX\) gene to search for the reported Pax6-binding sequences and to identify regulatory elements that bind to transcription factors.

The amount of \(FucT-IX\) mRNA in the telencephalic cortex of the wild type was 3-fold that of the homozygote (Fig. 6A). \(FucT-IV\), another member of the \(\alpha_3\)FucT family, was also expressed in the telencephalic cortex, but the expression level of its transcript was not affected by the \textit{Pax6} mutation (Fig. 6B). \(\alpha_3\)FucT activity toward PA-glycan in the wild type was 6-fold that of the homozygote (Fig. 4A). It is likely that the \(\alpha_3\)FucT activity measured in this study includes both \(FucT-IX\) and \(FucT-IV\) activities. The difference between the wild type and homozygote in the activities on glycolipid was less than that on PA-glycan (Fig. 4, A and B). This finding is consistent with the result obtained using recombinant enzymes; \(FucT-IX\) acts on PA-glycan more efficiently than \(FucT-IV\), compared with their actions on glycolipid (Table 1). The net increase of \(FucT-IX\) activity of the wild type compared with the homozygote, therefore, must be more than 6-fold. A 3-fold increase in the expression level of the transcript resulted in at least a 6-fold increase in the enzyme activity. This result was probably due to both increases of a translation rate and a turnover time of the transcript. Similarly, it is likely that the increase in the enzyme activity, which was at least 6-fold more, increased the production of the epitope, which resulted in the expression of the Lewis x epitope on the CSPG in the wild type (Fig. 2A). Thus, the immunohistochemical detection of Lewis x epitope in the wild type (Fig. 1A) may be attributable to the appearance of the epitope on the CSPG.

It has been reported that in the E14.5 mouse forebrain cortex, no obvious change in Lewis x immunoreactivity was observed between the \textit{Pax6} mutant and the wild type (16). A possible explanation is that the \textit{Pax6} target genes in the cerebral cortex may differ between the mouse and rat and that \textit{Pax6} does not regulate the expression of either \(FucT-IX\) or Lewis x in the mouse. It is also possible that the differences in the results between this and previous studies may be due to the different antibody specificity of anti-Lewis x antibodies used. In our study, a similar variability in antibody specificity was also observed in \textit{Pax6} mutant rat embryos. In the telencephalon of E13.5 \textit{Pax6} mutant rat embryos, Lewis x expression was not observed in the immunohistochemistry (Figs. 1D and 5F), whereas Lewis x glycolipids were detected in the HPTLC immunostaining (Fig. 2C). In support of this notion, different results were also obtained in other similar studies using different anti-Lewis x antibodies (e.g. Refs. 21, 33, 67, and 68). The exact specificity of the antibodies is likely to depend on the carrier of the Lewis x epitope; i.e. whether the epitope is on the protein or lipid, and the length, type, and modification of the core glycan. In our immunohistochemical analysis, the anti-Lewis x antibody (clone 73-30) appeared to preferentially detect the epitope on the CSPG rather than that on the glycolipid. However, the anti-Lewis x antibody used in other studies of Sey mice (clone RB11.2 in Ref. 16) may have preferentially detected the epitope on the glycolipid.

We showed that Lewis x-bearing CSPG was present in the telencephalic cortex in the wild type but not in the \textit{Pax6} mutant (Fig. 2). The core protein of the CSPG, however, remains to be identified. Therefore, we cannot exclude the possibility that \textit{Pax6} also regulates the expression of the core protein of the CSPG. Phosphacan/RPPTP-\(\beta\) is a CSPG expressed in the VZ of developing brain, binds with high affinity to the neural cell adhesion molecules, L1 and N-CAM, and the extracellular matrix protein tenascin-C (69, 70). This binding is mediated by N-linked glycans on the CSPG (71). In the mouse mesencephalon, it is suggested that the interaction between phosphacan and L1 is involved in the neuronal cell migration (72). Since only a subpopulation of phosphacan bears the Lewis x epitope (73), this finding suggests that the expression of Lewis x on phosphacan/RPPTP-\(\beta\) may affect cell migration by modulating the association of the CSPG with cell adhesion molecules. Similarly, the Lewis x-CSPG present in the telencephalic cortex may also be implicated in cell migration. In \textit{Pax6} mutant rat, defect in radial migration, which was shown to be not cell-autonomous, was observed in the later-born cortical precursor cells (9). This observation suggests that \textit{Pax6} affects a cortical environment. The disappearance of the Lewis x epitope from the CSPG, therefore, can be considered to be one of the molecular bases for the abnormal cortical environment in the \textit{Pax6} mutant.

It has been proposed that cell interactions via Lewis x epitope in pre-implantation embryos are mediated by the interaction of Lewis x glycans (26, 27). The interaction between Lewis x and Lewis x glycan is Ca\(^{2+}\)-dependent, and it has been suggested that it may cause autoaggregation of Lewis x-expressing cells (26, 27). It has also been demonstrated that cortical cells from the telencephalon aggregate with each other.
but segregate from striatal cells from the telencephalon (16, 19). This selective adhesion is Ca\textsuperscript{2+}-dependent (19) and is lost in Pax6 mutant (16). Taken together, it is considered that the selective adhesion of cells of the telencephalic cortex may be mediated, at least in part, by homophilic interaction of Lewis x glycan that is present on the CSPG. Lewis x glycan may act synergistically with R-cadherin, which has been shown to be involved in region-specific cell adhesion (74) and has been shown to disappear in the telencephalic cortex of Pax6 mutant (16). In pre-implantation embryos, both Lewis x glycan and E-cadherin are involved in the compaction process (75, 76). It has been suggested that glycan-glycan interaction takes place more rapidly than other species of intermolecular interactions and, although highly specific, is weaker than other interactions (77). The Lewis x epitope on the CSPG may act in the initial step of the cortex-specific cell adhesion in the telencephalon.

Leukocyte adhesion deficiency type II (LAD II) is a rare inherited disease caused by a metabolic disorder of GDP-fucose that results in hypofucosylation of glycoconjugates (reviewed in Refs. 78 and 79). LAD II is characterized by recurrent infections and leukocytosis and patients are reported to exhibit mental retardation and numerous facial abnormalities. These symptoms suggest that fucose-containing glycoconjugates, including Lewis x-carrying molecules, may play important roles in craniofacial morphogenesis and the development of brain function. It is well known that the Pax6 mutant mice and rats show craniofacial defects, i.e. the small eye phenotype in heterozygotes and the absence of eyes and nose in homozygotes (80, 81). It has been reported that in a family with inherited aniridia, only individuals with Pax6 mutation showed abnormalities in frontal lobe function (82). In patients with schizophrenia, it has been reported that the incidence of the high activity variant of the Pax6 promoter is higher in patients with the paranoid subtype than in the control (83). These observations may implicate a relationship between Lewis x epitope and Pax6 during development of the brain and face.

In conclusion, the findings of this study suggest that the expression of Lewis x epitope in the embryonic brain may be regulated by Pax6 via the expression of the FucT-IX gene. Based on these findings, we have now grasped a link between the regulatory gene and the carbohydrate epitope, which is expressed in a spatiotemporally regulated pattern.

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