Identification of the blood group Lewis[a] determinant in the oviducal mucins of *Xenopus tropicalis*

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Abstract The amphibian *Xenopus tropicalis* appears an increasingly appealing model for both genetic and developmental biology studies, compared to the related species *Xenopus laevis*. Study of the glycosylation pattern of its secreted glycoproteins revealed that this species synthesizes large amounts of Lewis[a] epitope, whereas this motif has previously only been identified in animals within the primate lineage. The use of ^{1}H-nuclear magnetic resonance spectroscopy enabled us to resolve the sequence of three Lewis[a]-bearing *O*-linked glycans associated with oviducal secretions, out of which one contained the novel sequence Gal(β1–3)GlcNAc(β1–6)GalNAc-ol. These structural data suggested the emergence of an α1,4-fucosyltransferase activity in animals outside the primate lineage. On this basis, the screening of a *X. tropicalis* GenBank database with human Lewis-fucosyltransferase sequences revealed the occurrence of a putative fucosyltransferase gene that presented an unusual acceptor motif.

Key words: Lewis[a] antigen; *O*-Glycan; Mucin; Fucosyltransferase; *Xenopus tropicalis*

1. Introduction

Previous structural studies have shown that the mucins, which are the major constituents of the amphibian egg jelly coats, possess species-specific glycanic chains. The characterization of several hundred novel *O*-glycosidic chains established the occurrence of complex panels of original glycosyltransferase activities in these organisms [1]. Most of these activities, such as the Kdn:fucosyltransferase or Fuc:fucosyltransferase activities, do not fit in any family of yet described glycosyltransferase. Similarly, no data permit to evaluate if glycosyltransferases involved in synthesis of ubiquitous determinants such as Lewis[y] or A blood group antigens in amphibians are somehow related to the mammalian enzymes, or originate from convergent evolution of their coding genes. Studies of glycosyltransferases from most amphibian species are greatly hampered by the lack of available molecular data. For several decades, the *Xenopus laevis* species has been extensively used as a model for all developmental sciences. However, neither *X. laevis* nor other amphibian species has provided the complementary powerful insights permitted by extensive genetic analyses. The main reason is that *X. laevis* is a pseudotetraploid, as a result of an additional genome duplication (relative to other vertebrates) about 30 millions years ago [2], which brings into the system a level of redundancy that makes genetic studies virtually impossible. In contrast, *Xenopus tropicalis* is the only diploid species of its genus. It shares all the advantages of *X. laevis* but its diploid status makes genetic studies possible. Among other advantages it has the shortest generation time of all amphibians [3], which enables faster generation of stable transgenic lines. In addition, because of the close relationship of *X. tropicalis* and *X. laevis*, many sequences between the two species are highly conserved. All of these features make *X. tropicalis* an exceptional vertebrate model system where classical embryological approaches can be combined with modern molecular approach [4].

Here, we report the first attempt to describe the glycosylation potentialities of this newly developed model. Surprisingly, *X. tropicalis* was shown to synthesize Le[a]-containing mucin-type *O*-glycans, whereas it was previously believed that synthesis of this motif in the animal kingdom was restricted to primates. Concomitantly, we observed the presence in *X. tropicalis* of a putative fucosyltransferase gene that comprises an original acceptor motif.

2. Materials and methods

2.1. Isolation and characterization of oligosaccharide alditols

Jelly coat mucin was extracted from eggs by gentle agitation in Dulbecco's phosphate-buffered saline (Sigma)/10 mM EDTA/1 mM phenylmethylsulfonyl fluoride/0.5% β-mercaptoethanol at 4°C overnight. The mixture was centrifuged at 4000 rpm for 15 min, the supernatant was then dialyzed for 72 h against water and finally freeze-dried. This material was subjected to alkaline reductive degradation in 100 mM NaOH containing 1.0 M $NaBH_4$ at 37°C for 72 h. The reaction was stopped by the addition of Dowex 50×8, 25–50 mesh, H^+ form (Bio-Rad) at 4°C until pH 6.5, and after evaporation to dryness, boric acid was repetitively distilled as methyl ester in the presence of methanol. Total material was subjected to cationic exchange chromatography on Dowex 50×2, 200–400 mesh, H^+ form (Bio-Rad) to remove residual peptides. The oligosaccharidic fraction was then purified on a TSK HW-40C (Toyo-Pearl) column. Oligosaccharide alditols were fractionated by high performance liquid chromatography (HPLC) on a primary amine-bonded silica column (Supelcosyl, LC-NH_2, 4.6×250 mm, Supelco, Bellefonte, CA, USA) using a mixture of acetonitrile/H_2PO_4K 30 mM/H_2O (75/0/25 to 50/50/0 in 60 min) with a flow rate of 1 ml/min. Oligosaccharides were detected by UV spectroscopy at 206 nm using a LDC variable-wavelength detector (Spectra Monitor D, Milton Roy, Riviera Beach, FL,

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USA) connected to a Spectra-Physics Model 4100 computing integrator.

2.2. Nuclear magnetic resonance (NMR) spectroscopy

The NMR experiments were performed on a Bruker® ASX400 spectrometer equipped with a 5 mm $^{1}H/^{13}C$ mixed probe head, operating in the pulse Fourier transform mode controlled by an Aspect 3000 computer. Each oligosaccharide was dissolved in 400 μl $^{2}H_2O$ after three exchanges with $^{2}H_2O$ at pD 7 (99.97% atom ^{2}H, Eurisotop, CEA group, Gif-sur-Yvette, France) and intermediate lyophilizations. The oligosaccharides were analyzed at 300 K. The chemical shifts (δ) were referenced to internal acetone ($\delta^{1}H = 2.225$ and $\delta^{13}C = 31.55$ ppm in the condition used). Two-dimensional homonuclear (COSY90, one relayed COSY and double relayed COSY) experiments were performed using standard Bruker® pulse programs (cosy, cosyr1, cosyr2). The main pulses and variable delays were optimized for each pulse program and sample.

2.3. Blot analysis

Mucin samples from *X. laevis* and *X. tropicalis* were solubilized overnight at 4°C in 6 M urea to a final concentration of 1 mg/ml. Solutions were diluted (1/40 to 1/1) in Tris-buffered saline (0.01 M Tris–HCl, 0.9% NaCl, pH 7.5) and spotted on a pre-wetted nitrocellulose membrane. The membrane was either revealed for carbohydrate by periodic acid Schiff reaction as previously described [5] or blotted with monoclonal anti-Le^{a} antibody. Antibody was produced from the ATCC hybridoma clone CF4-C4, by GlycoNex, Taiwan, as a generous gift. It was detected owing to a horseradish peroxidase-coupled secondary antibody.

3. Results and discussion

Both neutral and acidic oligosaccharide alditols released from the mucin by reductive β-elimination were fractionated by normal-phase HPLC (Fig. 1). Thirteen fractions were finally collected, out of which four (fractions **1**–**4**) contained non-carbohydrate material and two (fractions **11** and **13**) contained too complex mixtures of oligosaccharides to be analyzed. Of the remaining fractions, four (fractions **5**, **9**, **10** and **12**) were composed of mixtures of two or three oligosaccharide alditols that were analyzed as such, whereas the others (fractions **6**–**8**) contained single compounds. Oligosaccharides will be referred to according to the labeling of the HPLC fractions out of which they were isolated, some completed by A, B or C for mixtures of compounds (**5A**, **5B**, **5C**, **9A**, **9B**, **10A**, **10B**, **12A** and **12B**). Their structures have been entirely resolved through one- and two-dimensional ^{1}H-^{1}H NMR experiments and are summarized in Fig. 2. ^{1}H-NMR chemical shifts of each compound are summarized in Table 1.

Monosaccharide units were identified on the basis of the measurement of vicinal coupling constants, which discriminated the α,β-galacto and the α,β-gluco configurations, and GalNAc and GlcNAc were distinguished from Gal and Glc according to the downfield shift of their H-2 atom resonance. The substitution pattern of GalNAc-ol was easily inferred from the resonance position of its H-2, H-3, H-5 and H-6,6′ signals [6]. In particular, GalNAc-ol residues of all compounds showed H-2 chemical shift values between 4.253 and 4.404 ppm and H-3 values between 3.987 and 4.100 ppm, establishing that they were all substituted in C-3 positions. The observation of a single H-6/H-6′ signal at 3.64–3.66 ppm for compounds **5C**, **6** and **7** established that they were not substituted in C-6 positions. In contrast, for compounds **5A**, **5B**, **8**, **9A**, **9B** and **10A**, the marked deshielding of their H-5 signals at 4.130–4.280 ppm and of their H-6/H-6′ signals to average chemical shifts values of 3.93/3.68 ppm established that they were substituted in C-6 positions by β-GlcNAc residues. Then, compounds **10B**, **12A** and **12B** showed H-5 signals between 4.131 and 4.242 ppm and H-6/H-6′ signals with average chemical shifts values of 3.85/3.49 ppm, which were very distinctive of NeuAc or Kdn substitutions in C-6 positions.

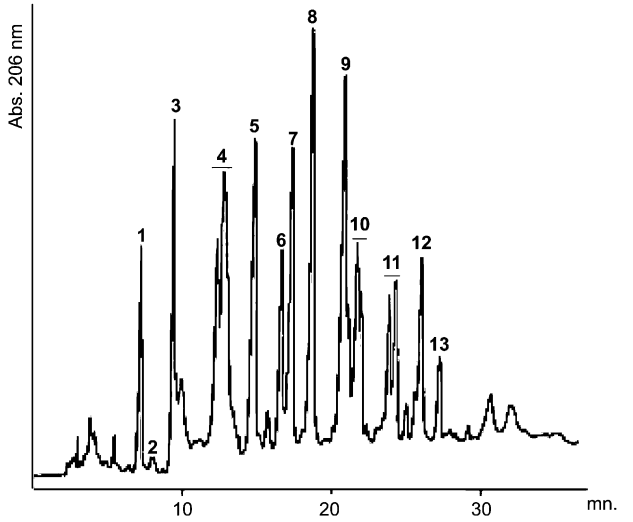

Fig. 1. HPLC profile of the oligosaccharides released by mild alkali treatment. Material was eluted from a primary amine-bonded silica column by H_2PO_4K gradient in a mixture of water and acetonitrile (CH_3CN/H_2PO_4K 30 mM/H_2O, 75/0/25 to 50/50/0 in 60 min).

The structures of carbohydrate appendages on C-3, and in some cases C-6, positions of the GalNAc-ol units from all compounds were determined owing to known structural reporter groups. Considering that the NMR values of one branch do not influence those of the other branch, their structures can be established independently. So, characteristic structural reporter groups of all described moieties are given in Table 2. Analysis of each compound revealed that most of them presented a well-known structure. In particular, compounds **5A**, **5B**, **5C** and **10B** are found in most mucins, and have already been extensively described [6]. Then, compounds **7**, **8**, **9B**, **10A** and **12B** were previously identified among the material released from the oviducal mucins of *Bufo bufo* and *Bufo arenarum* [7,8]. For this reason we will not describe these compounds again.

In contrast, compounds **6**, **9A** and **12A** showed original structures, and are characteristic of the *X. tropicalis* species. Indeed, these compounds exhibited NMR parameters typical of the presence of the immuno group Lewis^{a} determinant. H-1 and H-5 atom resonances of the α-fucose unit present in these three compounds are observed at δ 5.00–5.01 and δ 4.86–4.87 ppm, which are typical of the Lewis^{a} determinant [6], whereas the corresponding resonances for the immuno group Lewis^{x} determinant are found at δ 5.13–5.14 and δ 4.81–4.85 [9]. Moreover, the set of the β-GlcNAc H-2, H-3 and H-4 resonances, at δ 3.97, 4.09 and 3.73 respectively, is also typical of the Lewis^{a} determinant, compared with the values at δ 3.98, 3.90 and 3.95 observed for the Lewis^{x} determinant. In compound **6**, a specific shift of the GalNAc-ol H-1,1′ signals to δ 3.60–3.62 ppm confirmed the presence of the disaccharidic sequence GlcNAc(β1–3)GalNAc (Fig. 3) [9]. Compound **12A** presented identical NMR parameters except for the specific shifts for the GalNAc-ol H-6 and H-6′ signals that are characteristic of a substitution of the GalNAc-ol unit in the C-6

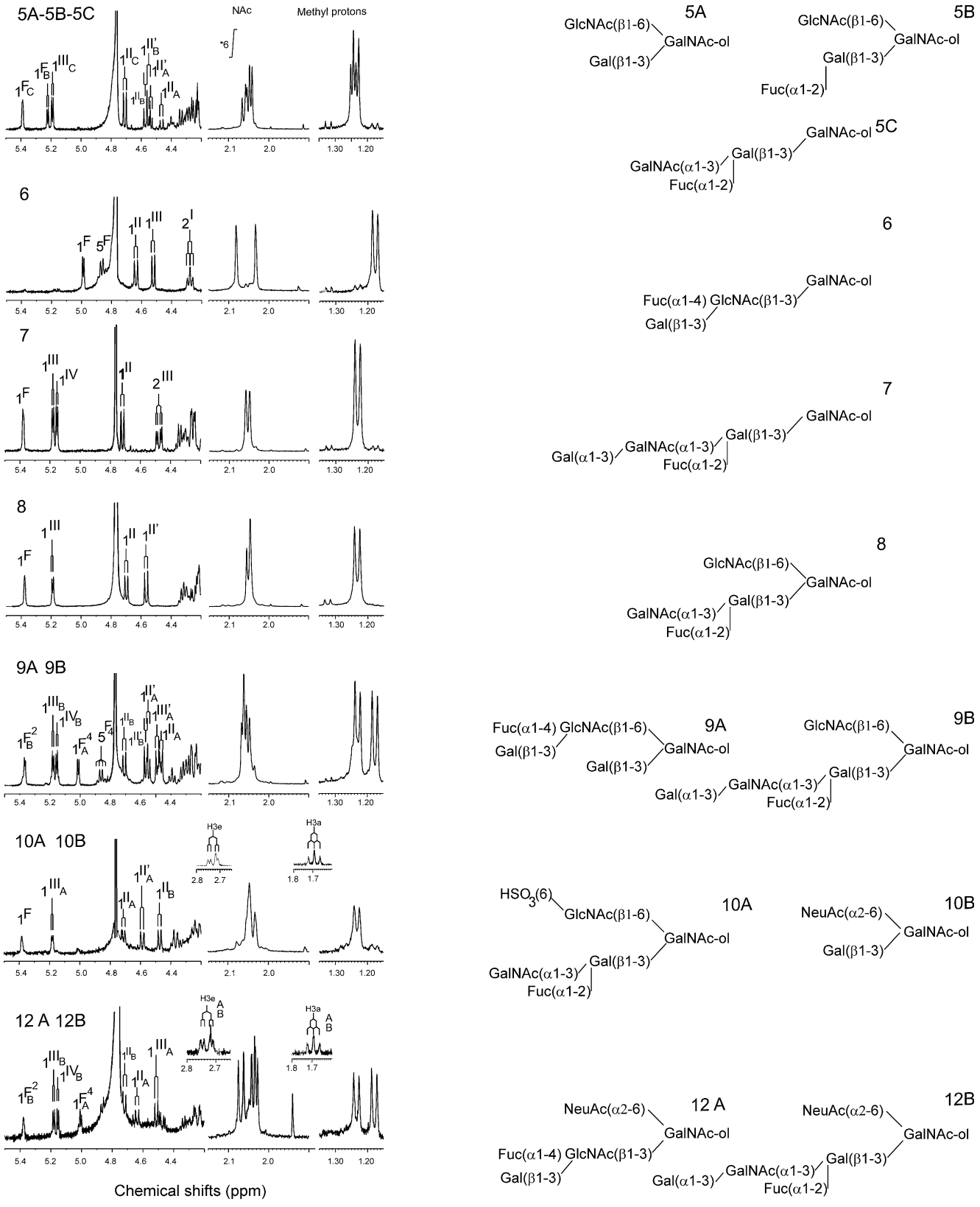

Fig. 2. Summary of structures of *O*-glycans isolated from oviducal secretions of *X. tropicalis*, with their corresponding ^{1}H-NMR spectra.

position by a residue of NeuAc. NeuAc was distinguished from Kdn which is commonly observed in amphibian *O*-glycans owing to its characteristic H-3 ax,eq chemical shift values and to the presence of a NAc signal at δ 2.033 ppm. Altogether, these data clearly established the sequence of compounds **6** and **12A** (Fig. 2).

Although **9A** and **9B** were analyzed in mixture, most of their ^{1}H-NMR signals were assigned. On the basis of NMR

Table 1
^{1}H NMR chemical shifts (ppm) of the oligosaccharide alditols isolated from oviducal secretions of *X. tropicalis*

		5A	5B	5C	6	7	8	9A	9B	10A	10B	12A	12B	X
GalNAc-ol I	H-1	3.75	3.780	3.79	3.620	3.800	3.780	3.787	3.78	3.79	3.779	3.60	3.780	3.610
	H-1′	3.75	3.780	3.79	3.62	3.800	3.780	3.730	3.78	3.79	3.725	3.60	3.780	3.590
	H-2	4.391	4.404	4.301	4.278	4.300	4.290	4.391	4.303	4.297	4.379	4.253	4.296	4.244
	H-3	4.060	4.084	4.095	3.999	4.100	4.083	4.060	4.088	4.076	4.058	3.987	4.093	3.978
	H-4	3.468	3.501	3.599	3.573	3.598	3.580	3.461	3.586	3.611	3.536	3.636	3.613	3.634
	H-5	4.280	4.252	4.118	4.126	4.118	4.223	4.280	4.216	4.198	4.243	4.131	4.181	4.161
	H-6	3.930	3.930	3.660	3.640	3.650	3.928	3.926	3.930	3.932	3.858	3.849	3.849	3.814
	H-6′	3.685	3.709	3.660	3.640	3.650	3.705	3.678	3.705	3.712	3.488	3.493	3.493	3.474
	NAc	2.066	2.054	2.047	2.033	2.048	2.048	2.036	2.048	2.046	2.048	2.027	2.042	2.023
Gal(β1–3) II	H-1	4.464	4.572	4.709	–	4.719	4.680	4.464	4.706	4.711	4.473	–	4.724	–
	H-2	3.561	3.674	3.896	–	3.907	3.891	3.560	3.901	3.911	3.571	–	3.919	–
	H-3	3.672	3.873	4.017	–	4.051	4.013	3.675	4.045	4.015	3.668	–	4.046	–
	H-4	3.901	3.923	4.224	–	4.242	4.220	3.903	4.235	4.209	3.894	–	4.232	–
GlcNAc(β1–3) II	H-1	–	–	–	4.637	–	–	–	–	–	–	4.638	–	4.645
	H-2	–	–	–	3.973	–	–	–	–	–	–	3.967	–	3.981
	H-3	–	–	–	4.097	–	–	–	–	–	–	4.093	–	3.897
	H-4	–	–	–	3.728	–	–	–	–	–	–	3.743	–	3.950
	H-5	–	–	–	3.592	–	–	–	–	–	–	3.580	–	3.603
	H-6	–	–	–	4.012	–	–	–	–	–	–	4.002	–	4.020
	H-6′	–	–	–	3.845	–	–	–	–	–	–	3.855	–	3.858
	NAc	–	–	–	2.082	–	–	–	–	–	–	2.075	–	2.066
GlcNAc(β1–6) II′	H-1	4.538	4.551	–	–	–	4.565	4.548	4.564	4.588	–	–	–	–
	H-2	3.707	3.716	–	–	–	3.723	3.908	3.722	3.735	–	–	–	–
	H-3	3.540	3.540	–	–	–	3.541	4.048	3.54	3.556	–	–	–	–
	H-4	3.460	3.460	–	–	–	3.457	3.729	3.460	n.d.	–	–	–	–
	H-5	3.460	3.460	–	–	–	3.460	3.557	3.460	3.660	–	–	–	–
	H-6	3.934	3.934	–	–	–	3.940	3.988	3.941	4.368	–	–	–	–
	H-6′	3.743	3.743	–	–	–	3.750	3.849	3.745	4.233	–	–	–	–
	NAc	2.066	2.058	–	–	–	2.056	2.068	2.062	2.058	–	–	–	–
Gal(β1–3) III, III′ or Gal(β1–4)	H-1	–	–	–	4.520	–	–	4.487	–	–	–	4.512	–	4.444
	H-2	–	–	–	3.480	–	–	3.480	–	–	–	3.483	–	3.494
	H-3	–	–	–	3.635	–	–	3.623	–	–	–	3.636	–	3.660
	H-4	–	–	–	3.889	–	–	3.882	–	–	–	3.890	–	3.902
GalNAc(α1–3) III	H-1	–	–	5.190	–	5.180	5.188	–	5.178	5.181	–	–	5.180	–
	H-2	–	–	4.246	–	4.477	4.288	–	4.476	4.253	–	–	4.475	–
	H-3	–	–	3.928	–	4.028	3.932	–	4.037	3.929	–	–	4.027	–
	H-4	–	–	4.021	–	4.264	4.021	–	4.263	4.023	–	–	4.264	–
	H-5	–	–	4.156	–	4.184	4.158	–	4.184	4.165	–	–	4.186	–
	H-6,6′	–	–	3.780	–	3.790	3.770	–	3.790	3.770	–	–	3.790	–
	NAc	–	–	2.041	–	2.058	2.047	–	2.056	2.048	–	–	2.042	–
Gal(α1–3) IV	H-1	–	–	–	–	5.154	–	–	5.154	–	–	–	5.153	–
	H-2	–	–	–	–	3.831	–	–	3.830	–	–	–	3.830	–
	H-3	–	–	–	–	3.755	–	–	3.755	–	–	–	3.755	–
	H-4	–	–	–	–	3.987	–	–	3.990	–	–	–	3.992	–
	H-5	–	–	–	–	4.184	–	–	4.184	–	–	–	4.186	–
	H-6,6′	–	–	–	–	3.790	–	–	3.790	–	–	–	3.790	–
Fuc(α1–2)	H-1	–	5.222	5.390	–	5.378	5.376	–	5.365	5.383	–	–	5.377	–
	H-2	–	3.804	3.803	–	3.810	3.810	–	3.810	3.800	–	–	3.810	–
	H-3	–	3.911	3.800	–	3.810	3.810	–	3.810	3.800	–	–	3.810	–
	H-4	–	3.827	3.817	–	3.810	3.810	–	3.803	3.814	–	–	3.800	–
	H-5	–	4.275	4.337	–	4.340	4.323	–	4.327	4.328	–	–	4.334	–
	H-6	–	1.243	1.235	–	1.232	1.231	–	1.230	1.233	–	–	1.235	–
Fuc(α1–4) or Fuc(α1–3)	H-1	–	–	–	4.988	–	–	5.012	–	–	–	5.004	–	5.132
	H-2	–	–	–	3.796	–	–	3.799	–	–	–	3.795	–	3.700
	H-3	–	–	–	3.889	–	–	3.887	–	–	–	3.790	–	3.902
	H-4	–	–	–	3.795	–	–	3.791	–	–	–	3.787	–	3.792
	H-5	–	–	–	4.865	–	–	4.861	–	–	–	4.868	–	4.818
	H-6	–	–	–	1.178	–	–	1.176	–	–	–	1.179	–	1.176
NeuAc(α2–6) or Kdn(α2–6)	H-3 ax	–	–	–	–	–	–	–	–	–	1.692	1.692	1.692	1.652
	H-3 eq	–	–	–	–	–	–	–	–	–	2.728	2.731	2.731	2.676
	H-4	–	–	–	–	–	–	–	–	–	3.666	3.670	3.670	3.603
	NAc	–	–	–	–	–	–	–	–	–	2.033	2.033	2.033	–

An additional compound containing Le^x epitope (**X** = Gal(β1–4)[Fuc(α1–3)]GlcNAc(β1–3)[Kdn(α2–6)]GalNAc-ol) was inserted for reference.

parameters from previously analyzed compound **9B** [7] the resonances belonging to **9A** and **9B** were easily distinguished (Fig. 3). The δ values of GalNAc-ol H-1,1′ demonstrated the absence of a GlcNAc(β1–3)GalNAc-ol sequence, which classified **9A** (and **9B**) as belonging to the Gal(β1–3)GalNAc-ol core structure. The attachment of an α-Fuc residue via α-1,4 linkage to GlcNAc II′ is established from the chemical shifts of Fuc H-1 at δ 5.012 and H-5 at δ 4.861, and GlcNAc H-3 at δ 4.048 and H-4 at δ 3.729 [6]. The two β-Gal units present in compound **9A** occur in non-reducing terminal position, as

Table 2
Characteristic structural reporter groups used for structural determination of oligosaccharidic motifs, classified according to their position on the GalNAc-ol residue

	Compounds	Characteristic structural reporter groups
Branches in C-3 position of GalNAc-ol		
Gal(β1–3)	**5A**, **9A**, **10B**	α-Gal H-2, H-3, H-4
Fuc(α1–2)Gal(β1–3)	**5B**	β-Gal H-1 α-Fuc H-1, H-5
GalNAc(α1–3)[Fuc(α1–2)]Gal(β1–3)	**5C**, **8**, **10A**	α-Fuc H-1, H-5 β-Gal H-1, H-4 α-GalNAc H-1, H-2
Gal(α1–3)GalNAc(α1–3)[Fuc(α1–2)]Gal(β1–3)	**7**, **9B**, **12B**	α-Fuc H-1, H-5 α-Gal H-1, H-2, H-3, H-4 α-GalNAc H-2, H-3, H-4 β-Gal H-1, H-4
Gal(β1–3)[Fuc(α1–4)]GlcNAc(β1–3)	**6**, **12A**	α-Fuc H-1, H-5 β-GlcNAc H-3, H-4 GalNAc-ol H-1,1′
Branches in C-6 position of GalNAc-ol		
GlcNAc(β1–6)	**5A**, **5B**, **8**, **9B**	β-GlcNAc H-2, H-3, H-4
HSO_3(6)GlcNAc(β1–6)	**10A**	β-GlcNAc H-6,6′
NeuAc(α2–6)	**10B**, **12A**, **12B**	α-NeuAc H-3ax, H-3e GalNAc-ol H-6,6′
Gal(β1–3)[Fuc(α1–4)]GlcNAc(β1–6)	**9A**	α-Fuc H-1, H-5 β-GlcNAc H-3, H-4 GalNAc-ol H-6,6′

inferred from the set of their H-2, H-3 and H-4 atom resonances (Fig. 3). The presence of Gal(β1–3)GlcNAc (type 1 chain) directly β-1,6-linked to GalNAc is here reported for the first time in a mucin, whatever its origin.

Out of the 12 complex *O*-glycans we have sequenced, three included the Lewis[a] trisaccharidic motif. In the animal kingdom, synthesis of the Lewis[a] determinant involves the expression of two fucosyltransferases, namely FUT3 ant FUT5, be-

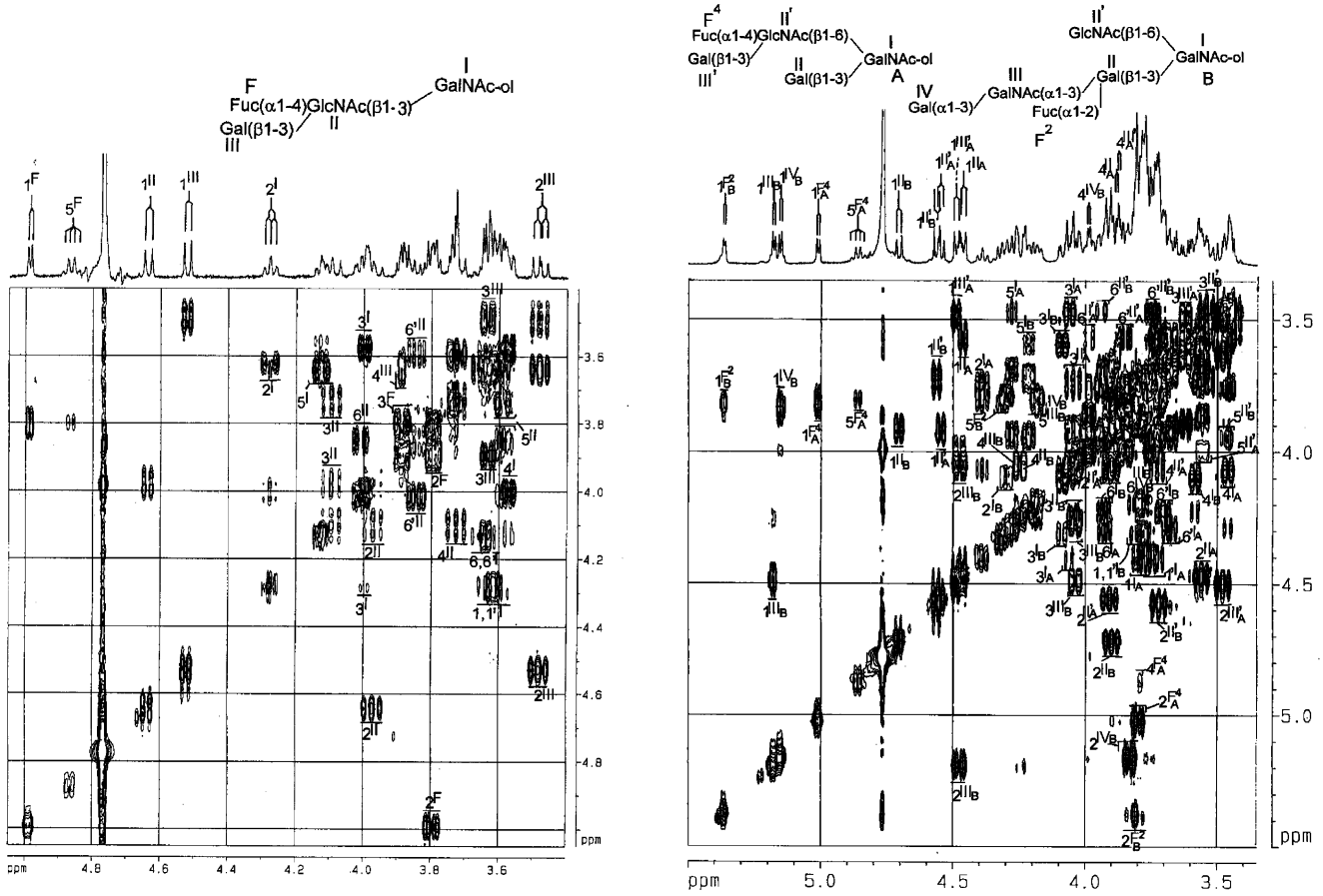

Fig. 3. ^{1}H-^{1}H two-dimensional COSY 90 spectra of compounds **6** (left) and **9A-9B** (right).

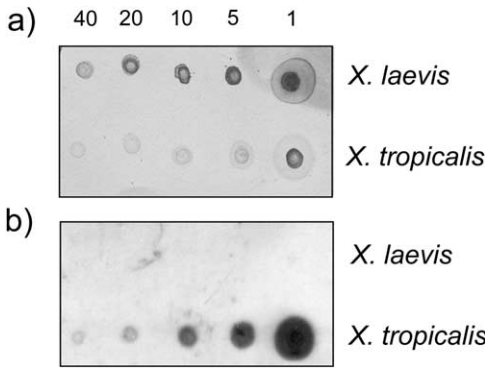

Fig. 4. Reactivity of *Xenopus* mucins toward anti-Le^a monoclonal antibody. Dot blot analysis on nitrocellulose of diluted solutions (from 1/40 to 1/1) of *X. laevis* and *X. tropicalis* mucins either (a) revealed for carbohydrates with periodic acid Schiff reaction or (b) probed with anti-Le^a monoclonal antibody.

longing to the FUT-Lewis family [10]. Within this family, another gene, FUT6, encodes a protein involved in the synthesis of the Le^x/Le^y determinant. Site-directed mutagenesis experiments proved that amino acids of the 'acceptor motif' in the NH_2-terminal region of the protein are required for the fucose substitution on peripheral GlcNAc, in either the α-1,3 or the α-1,4 position [11]. The short consensus motif HHRD/E was shown to be associated exclusively with α-1,3 linkage, whereas HHWD is associated with both α-1,3 and α-1,4 linkages. So far, within the animal kingdom, α-1,4 fucosyltransferase activity has only been uncovered in primates. This was suggested by the detection of Lewis^a determinant substituting glycoproteins and glycolipids only in man and demonstrated by the characterization of FUT3 and FUT5 exclusively in man and chimpanzee [12]. Recently, an orthologous gene of FUT3, FUT3-rh, was also cloned from an old world monkey [13]. The presence of two genes which directed the α-1,4 fucosyltransferase activity suggests that among animals this function has appeared through two successive duplications of an ancestral Lewis gene, which occurred between the great mammalian radiation and the separation of higher apes and man [13]. In the present report, the finding of a Lewis^a epitope for the first time outside the primates suggests that an α-1,4 fucosyltransferase activity has appeared in the amphibian species *X. tropicalis* independently of the α-1,4 genes in primates, and presumably through a convergent evolution phenomenon. Previous studies of the glycosylation pattern of the closely related species *X. laevis* [14] did not reveal the presence of any glycan resulting from an α-1,4 fucosyltransferase activity, including Le^a determinant, suggesting that this activity is specific to *X. tropicalis* species. This point was further assessed in the present study by testing the reactivity of oviducal mucins isolated from both *Xenopus* species toward a monoclonal anti-Le^a antibody. As shown Fig. 4, the *X. laevis* sample was non-reactive toward this antibody at every tested dilution, whereas *X. tropicalis* reacted strongly with the antibody, confirming that the Le^a motif is exclusively synthesized by *X. tropicalis*. It is noteworthy that *X. laevis* does not synthesize the Gal-(β1–3)GlcNAc disaccharide that may be used as acceptor substrate by an α-1,4 fucosyltransferase for the synthesis of Le^a [14]. In other amphibian species α-1,4 fucosylation was also shown to occur on diverse substrates such as GlcNAc-(β1–3)GlcNAc or Kdn [1], which suggests the occurrence of a yet unknown polymorphism of α-1,4 fucosyltransferase activities in the animal kingdom.

From these observations, we developed a strategy based on screening of expressed sequence tag (EST) databanks and polymerase chain reaction amplification in order to identify putative fucosyltransferase genes involved in Lewis^a determinant synthesis. Screening of α-1,3/4 fucosyltransferase sequences in GenBank with human sequences as probes revealed in *X. laevis* two *FUT* ESTs (accession numbers BJ038553 and BJ036679). We cloned and sequenced the corresponding cDNA named thereafter *FUT Lewis 1* (accession number AY278679) and *FUT Lewis 2* (accession number AY278677). As no genomic information is available on these sequences, we could not test whether this pair of genes is the result of allopolyploidy or tandem duplication. In *X. tropicalis*, a true diploid species, no *Lewis-like FUT* gene was evidenced in EST GenBank database.

The putative proteins encoded by *X. laevis FUT Lewis 1* and *FUT Lewis 2* could comprise 349 and 347 amino acids respectively (Table 3). Unexpectedly, the acceptor motif of FUT Lewis 1 presented the original sequence HSRD that does not fit with the previously described sequences HHRD/E or HHWD for α-1,3 and α-1,3/4 activities respectively [11]. In contrast, FUT Lewis 2 comprised the classical conserved sequence HHRE as shown below:

Xla – FUT Lewis 1 SLYSEAAAVVL**HSRD**VCSS

Xla – FUT Lewis 2 SLYPSANAVII**HHRE**VYKN

Based on the conserved sequences of both *X. laevis* cDNA, we cloned in *X. tropicalis* a 841 bp long DNA segment (accession number AY278678). The partial peptide sequence encoded by this DNA fragment is very close to *X. laevis* FUT Lewis 1 and with the same putative acceptor motif. Full characterization of *X. laevis* and *X. tropicalis* genes still requires expression of the genes in a heterologous system as well as the use of appropriate substrates to test their activities.

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Table 3
FUT Lewis-like sequences detected in *Xenopus* species

	FUT name	Nature	Protein length (aa)	EST accession number	Sequence accession number	Tissue/stage	Reference
X. laevis	FUT Lewis 1	EST	349	BJ038553	AY278679	Neurula	T. Shin-I, unpublished
	FUT Lewis 2	EST	347	BJ036679	AY278677	Neurula	T. Shin-I, unpublished
X. tropicalis	FUT Lewis 1	genomic	>280		AY278678		Present work

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