Ubiquitous Expression of the $\alpha 1(XIX)$ Collagen Gene (*Col19a1*) during Mouse Embryogenesis Becomes Restricted to a Few Tissues in the Adult Organism^{*}

(Received for publication, November 26, 1996, and in revised form, February 21, 1997)

Hideaki Sumiyoshi, Kazuhito Inoguchi, Mohammed Khaleduzzaman, Yoshifumi Ninomiya, and Hidekatsu Yoshioka‡

From the Department of Molecular Biology and Biochemistry, Okayama University Medical School, Okayama 700, Japan

Type XIX collagen is a poorly characterized member of the fibril-associated collagens with an interrupted triple helices (FACIT) class of collagen molecules. As a first step toward elucidating its function, we have isolated full size cDNA clones from the mouse $\alpha 1(XIX)$ collagen gene (Col19a1) and established its pattern of expression in the developing embryo and adult organism. Col19a1 transcripts can be detected as early as 11 days of gestation and in all embryonic tissues, except the liver, of an 18-day postcoitum mouse. In contrast, only a few adult tissues, brain, eye, and testis, seem to accumulate Col19a1 mRNA. Col19a1 transcripts are at least 10 times more abundant in adult than fetal brain and significantly less in adult than fetal muscle and skin. Consistent with the RNA data, polyclonal antibodies for $\alpha 1(XIX)$ collagen reacted with a 150-kDa protein in the neutral salt extraction of adult mouse brain tissues. We therefore propose that type XIX collagen plays a distinct role from the other FACIT molecules, particularly in the assembly of embryonic matrices and in the maintenance of specific adult tissues.

Collagenous networks play a critical role in the morphogenesis of the embryo and in the maintenance of tissue architecture of adult tissues. The 19 genetically distinct types of collagen molecules are expressed in developmental and tissuespecific manners (1-4). The collagen superfamily can be classified into several subgroups according to the structural features of its members, the supramolecular aggregates that they form, and the structure of the gene that the proteins are encoded. One of the most interesting and less characterized collagen subgroups is the one of the so-called FACIT¹ (fibrilassociated collagens with interrupted triple helices) which includes types IX, XII, XIV, XVI, and XIX collagen. Available evidence suggests that FACIT may play an important role in providing tissue-specific molecular links between collagen fibrils and other extracellular matrix aggregates (5, 6). With the exception of types IX and XII, virtually nothing is known about the expression pattern of the other FACITs. Without this information, no function has yet been postulated for types XIV, XVI, and XIX collagen.

Type XIX collagen was originally discovered through cDNA cloning of RNA transcripts from the human rhabdomyosarcoma cell line RD (CCL 136) (7). The predicted polypeptide was found to contain 1,142 amino acid residues with a 23-residue signal peptide followed by the five collagenous (COL) domains, interspersed and flanked with the six non-collagenous (NC) domains (3, 4, 8). The coding region of the $\alpha 1(XIX)$ mRNA is small compared with the length of the entire transcript (10.4 kb), due to the presence of more than 5-kb long 3'-untranslated region. Additionally, an unusual number of splicing events appeared to occur in the RD cell line. More interestingly, the α 1(XIX) collagen gene (COL19A1) was located to human chromosome 6q12-q14, syntenic to the $\alpha 1(IX)$ collagen (COL9A1) and $\alpha 1(XII)$ collagen genes (COL12A1) (7, 9). Type IX collagen is found in tissues containing type II collagen, such as hyaline cartilage and the vitreous body of the eye. On the other hand, type XII collagen is found in dense connective tissues, such as tendons and ligaments, where type I is the major collagenous component. Aside from the rhabdomyosarcoma cell line, there is currently no information about the tissue distribution of type XIX collagen and, consequently, about its possible function.

The present study was designed to fill this gap of knowledge and to generate suitable reagents to eventually establish type XIX collagen function using the gene targeting approach in mouse embryonic stem cells. Accordingly, we isolated overlapping cDNA clones coding for the entire mouse $\alpha 1(XIX)$ chain; we examined the pattern of mRNA accumulation in the tissues of both the developing and adult mice; and we identified the $\alpha 1(XIX)$ protein in adult brain tissues.

MATERIALS AND METHODS

Isolation and Characterization of cDNA Clones—Polv(A)⁺ RNA was isolated from 18 d.p.c. mouse whole embryo tissue according to the standard protocol (10) and was used as a template for cDNA synthesis. Double-stranded cDNA was synthesized using random primers and then inserted into the λ gt10 vector using a commercial kit (Amersham Corp.). Three distinct mouse embryonic cDNA libraries were screened under low stringency conditions using probes specific for the human al(XIX) collagen (4, 7). Hybridizations carried out at 55 °C overnight in a mixture containing 5 \times SSC (1 \times SSC; 0.15 m NaCl, 0.015 m sodium citrate, pH 6.8), 1% N-laurylsarcosine, 50 µg/ml salmon sperm DNA, and ³²P-labeled probes after prehybridization which is the same solution without probes for 1 h. Isolation and purification of the positive clones were as described previously (7). Sequencing was carried out according to the dideoxy chain termination methods using an ABI 373S automatic sequencer. After sequencing validation of positive clones, a 0.6-kb gap in the α 1(XIX) coding sequence was resolved by amplifying an aliquot of reverse-transcribed embryonic cDNA using the polymerase chain technique (RT-PCR). Twenty microliters of reverse transcrip-

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

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AB000636.

[‡] Present address and to whom correspondence and reprints should be addressed: Dept. of Molecular Biology and Biochemistry, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700, Japan. Tel.: 81-86-235-7128; Fax: 81-86-222-7768; E-mail: mbb@ccews2. cc.okayama-u.ac.ip.

cc.okayama-u.ac.jp. ¹ The abbreviations used are: FACIT, fibril-associated collagens with interrupted triple helices; bp, base pairs; kb, kilobases; d.p.c., days postcoitum; RT-PCR, reverse transcription-polymerase chain reaction; COL, collagenous; NC, noncollagenous; MBP, maltose binding protein; PAGE, polyacrylamide gel electrophoresis.



FIG. 1. The domain structure of the mouse $\alpha 1$ (XIX) collagen chain deduced from nucleotide sequences of cDNA clones and their partial restriction map. Five overlapping cDNAs, ME1, ME2, ME12, ME14, and ME23, were isolated from 18 d.p.c. mouse whole embryo library. A clone, ME PCR, was generated from RNA of 18 d.p.c. mouse whole embryo using RT-PCR method. The alignment of these cDNAs was determined by restriction enzyme digestions and DNA sequencing. Amino acid sequence deduced from the cDNA sequence allowed the prediction of the domain structure of the mouse $\alpha 1$ (XIX) chain (*top* of the figure). Numbers of the amino acid residues in individual domains of COL1–5 and NC1–6 are shown in *parentheses*. Locations of the 16 cysteinyl residues are indicated by *closed circles*. Positions of the nine imperfections of the Gly-X-Y repeated structure are shown by *short bars above* the COL domains. The signal peptide of 23 amino acid residues is indicated by *thicker bar* at the amino terminus of the chain. Locations of 5' and 3' mouse probes used for the Northern blotting analysis (in Fig. 6) are indicated by *thin bars* with the *numbers 1* and 2 in *parentheses*.

tion reaction mixture (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol) containing 2 μ g of total RNA from 18 d.p.c. mouse embryo, 0.25 mM dNTP, 2 units of RNasin (Toyobo, Osaka), 400 ng of random hexamer, and 100 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) was incubated at 37 °C for 1 h, heated to 70 °C for 10 min, and quick-chilled on ice, and diluted with 80 μ l of water. To get appopriate RCR products, the nested PCR was performed. First PCR reaction was performed using 2 μ l of cDNA solution in 20 μ l of mixture containing 0.8 units of Tth DNA polymerase (Toyobo) using primer 1 (forward, 5'-GGAACTTAAAGACACATGCC-3', Fig. 2) and primer 2 (reverse, 5'-ATGTCTCCCAAGAGAGGAAG-3', Fig. 2), under the following condition: 94 °C for 1 min, 60 °C for 1 min, and 70 °C for 3 min for 35 cycles. Five microliters of the first PCR amplification product was used for the second PCR reaction with primer 3 (forward, 5'-CCTCAATGGACAAGATGGTT-3', Fig. 2) and primer 4 (reverse, 5'-TTCTACCAGGGATTCCTACAC-3', Fig. 2) under same condition. PCR products were subcloned in TA vectors (Invitrogen). The sequence was confirmed with both strands of two clones.

Northern Blot and RT-PCR Analysis—For Northern blotting analysis, approximately 3 or 5 μ g of poly(A)⁺ RNA was electrophoresed in 0.8% agarose gel under denaturing conditions, blotted onto Hybond N nylon filter (Amersham Corp.), and to hybridized species-specific probes (10). Sources of RNA included 18 d.p.c. mouse whole embryo, 5-week-old mouse brain, human rhabdomyosarcoma cell line (RD), human glioma cell line (U251MG), human infant skin fibroblast culture cells, and human adult skin fibroblast culture cells. The following mouse tissues and cultured cells were instead used for RT-PCR amplification: 11, 12, and 14 d.p.c. mouse whole embryo; different tissues from 18 d.p.c. mouse embryo and 5-week-old mouse; the human rhabdomyosarcoma cell lines RD, A204 and KYM1; the human glioma cell lines U251MG, U373MG, and A172; the human neuroblastoma cell lines TGW and NB1; and skin fibroblasts from mouse embryo and mouse adult and human infant and human adult. Single strand cDNA was synthesized as mentioned above. PCR was performed with 35 cycles for $\alpha 1(XIX)$ or 30 cycles for β actin using 0.8 units of Tth DNA polymerase at 94 °C for 1 min, at 60 °C for 2 min, and at 70 °C for 3 min. The nucleotide sequences for the primers used in these reactions are as follows: $\alpha 1(XIX)$, 5'-AACTGCCAGCAGCAATGTTG-3' (forward) and 5'-CAATCTTCTGGATTACATCT-3' (reverse); β actin, 5'-AAGAGAGGG-TATCCTGACCCT-3' (forward) and 5'- TACATGGCTGGGGTGTT-GAA-3' (reverse).

For competitive RT-PCR, $\alpha 1(XIX)$ collagen and β actin cDNA clones were modified using restriction enzymes to delete 51 bp in the former and 61 bp in the latter. These modified clones were then used as competitors in RT-PCR amplifications of various mouse samples. To determine the optimal condition, a series of RT-PCR reactions containing 2-fold serial dilutions of competitor (ranging from 1 pg to 1 fg/µl for $\alpha 1(XIX)$, and from 50 pg to 1.6 pg/µl for β actin) were first carried out (11). PCR was then performed described above except that 5 more cycles were added to each reaction. Aliquots of each amplification were electrophoresed in 2% or 2.5% agarose gels (Sigma) containing 0.5 µg/ml ethidium bromide; gels were photographed with Polaroid film (Polaroid type 667); photographs were quantitatively scanned using the NIH image software.

Preparation of Type XIX Collagen Antibodies-A recombinant $\alpha 1 (\rm XIX)$ NC1-COL1-NC2 peptide was made by expressing a cDNA fragment subcloned into EcoRI and PstI sites of pMALc2 (New England BioLabs Inc.) expression plasmid. Production of the MBP (maltose binding protein)- α 1(XIX)COL fusion proteins was induced by the addition of isopropyl- β -D-thiogalactopyranoside (final concentration of 0.3 mm) to the bacterial cultures incubated at 37 °C for 2 h. The cells were lysed by lysozyme treatment, and the fusion proteins were purified by affinity chromatography on an amylase column as describe by the manufacturer (New England Biolabs Inc.). After incubation of the fusion protein with Xa-specific peptidase, the $\alpha 1(XIX)COL$ recombinant protein was separated on 12.5% SDS-PAGE gel and isolated in a gel slice after Coomassie Brilliant Blue R (Merck, Darmstadt, Germany) staining (12). The gel slices containing approximately 500 μ g of protein in total were washed with phosphate-buffered saline, cut into the small pieces, emulsified with the equal volume of complete Freund's adjuvant (Difco), and then injected subcutaneously into a rabbit. Booster injections (200 μ g) were given with incomplete Freund's adjuvant (Difco) 3

						GCG	GGTG	CGGCI	AGCC	CTGT	CCGGI	ACTCO	CACC	GCGC	CTCC	CAGG	GCT	CTGA	CACC	AACCO	CAAGI	ATCT	GTGG	CAT	FTGT:	FTCC.	ATGG	TTAC	AAGG	CACA	98	
1	ATG M	AGG R	CAC H L	ACT T	GGC G	TCT S P	TGG W	AAA K	TTG L	TGG W	ACC T L	TGG W	GTG V M	ACA T S	ACC T I	TTC F	CTG L	CTT L	CCT P	GCA A	TGT C S	ACC T	TGT C S	CTG L V	ACA T	GTC V	AGG R	GAC D	AAG K	CCA P T	188	
31	GAA E	ACA T E	ACC T S		CCT P	ACA T I	CTG L	AGA R	ACA T I	GAG E	AGA R G	TAT Y H	CAA Q	*** * L	*** * T	*** * Y	GAT D	GAC D N	AGA R I	AAC N	aaa K	TCT S L	GAA E	TTG L V	TCA S S	GGC G	TTT F	GAT D	CTG L	GGA G	269	
58	GAA E D	AGC S	TTT F	GCT A S	CTG L	AGG R	CAT H R	GCG A	TTT F		GAA E	GGT G S	GAT D	aaa K	ACC T		TTC F	aag K	TTG L	GGA G	AGT S	GTG V A	CTT L	CTT L	ATC I	AGA R	GAC D	ACT T	GTT V I	aag K	359	
88	ATA I	TTT F	CCC P	AAA K	GGC G	CTT L	CCA P	GAG E	GAA E	TAC Y	GCT A S	ATA I V	GCT A	GTC V A	ATG M	TTT F	CGG R	GTA V	CGA R	AGA R	AGC S N	ACC T A	CAA Q K	aag K	GAA E	CGG R	TGG W	TTT F	TTA L	TGG W	449	
118	AAG K Q	ATT I V	TTA L	AAC N	CAG Q	CAG Q	AAT N	ATG M I	GCC A P	CAG Q	ATA I	тст s	GTA V I	GTG V	ATT I V	GAT D	GGT G	ACG T G	AAG K	AAG K	GTG V	GTA V	GAA E	TTT F	atg M	TTC F	CGA R Q	GGT G A	GCT A T	GAG E	539	NC 6
148	GGA G	GAT D	CTG L V	TTG L	AAC N	TAT Y	GTT V I	TTT F	AAG K R	AAT N	CGA R	GAA E	CTC L	CGT R	CCA P	CTC L	TTT F	GAT D	CGT R	CAA Q	TGG W	CAT H	AAA K	CTT L	GGC G	ATT I	GGC G S	GTG V I	CAA Q	TCC S	629	
178	CGA R Q	GTC V	CTT L I	TCT S	CTC L	TAT Y	ATG M	GAT D	TGC C	AAT N	TTA L	ATT I	GCA A	AGT S R	CGA R	CAC H Q	ACG T	GAA E D	GAA E	AAG K	AAT N D	TCT S T	GTG V	GAT D	TTT F	CAA Q H	GGA G	AGG R	ACA T	ATT I V	719	
208	ATT I	GCT A	GCA A T	CGA R	GCT A	TCA S	GAT D	GGC G	AAA K	CCT P	GTG V	GAT D	ATC I	gaa e	CTT L	CAC H	CAA Q	CTT L	AGA R K	ATC I	TAC Y		AAT N S	GCA A	AAC N	TTC F L	CTA L I	GCT A	GAA E Q	GAA E	809	
238	TCA S T	TGT C	TGC C	AAC N E	TTA L I	TCA S	ССТ Р D	ACT T	AAG K	TGT C	CCA P	GAG E	CAA Q	GAC D	GAC D G	TTT F	GGA G	AGT S N	ACT T I	ACT T A	TCA S	TCC S	TGG W	GGG G V	ACC T	TCT S A	AAC N H	ACT T A	GGT G S	aaa K	899	
268	ATG M	TCT S	TCA S	TAT Y	CTG L	CCA P	GGA G A	AAG K	CAG Q	GAA E	CTT L	AAA K	GAC D	ACA T Q		CAG Q	TGT	ATT I	CCA P	AAC N	AAG K	GAG E G	GAA E	GCA A	GGA G	TTA L	CCA P	GGA G	ACT T A	CTC L P	989	
298	AGA R G	TCC S	ATT I P	GGA G	CAT H Q	AAG K	GGC G	GAC D H	AAA K	GGA G	GAG E	CCG P	GGT G	GAA E	CAT H N	GGC G	TTA L	AAT N H	GGT G	ACT T A	CCA P	GGA G	TTA L F	CCT P	GGT G	CAA Q	aag K	GGA G	GAA E	CAA Q	1079	
328	GGA G	CTT L F	GAA E	GGC G	ATC I S	AAA K	GGA G	GAA E	ATT I T	GGT G	GAA E	AAG K	GGT G	GAA E	CCA P Q	GGA G	GCC A E	AAA K	GGA G	GAC D	TCT S P	GGT G A	CTG L	GAT D A	GGC G	CTC L	AAT N	GGA G	CAA Q E	GAT D N	1169	COL 5
358	GGT G	TTG L	AAA K	GGT G	GAC D	TCG S L	GGT G	CCT P	CAA Q H	GGT G	CCA P	CCT P	GGC G	CCA P	AAA K	GGA G	GAC D E	AAG K	GGT G	GAT D	ATG M T	GGA G	CCT P	CCA P	GGG G	CCA P	CCA P	GCC A	TTA L	ACT T P	1259	
388	GGC G	TCC S	ATA I L	GGA G	ATC I	CAA Q	GGT G	CCC P	CAG Q	GGT G	CCA P	CCT P	GGC G	aaa K	GAA E	GGC G	CAA Q	AGG R	GGA G	AGA R	CGA R	GGG G	AAA K	ACA T	GGA G	CCC P	CCA P	GGA G	AAT N K	CCA P	1349	
418	GGA G	CCT P	CCA P	GGA G	CCA P	CCC P	GGA G	CCT P	CCT P	GGG G	TTA L I	CAA Q	GGA G	CTA L I	CAG Q H	CAG Q	CCT P T	TTT F L	GGT G	GGA G	*** * Y	*** * Y	*** * N	TAT Y K	TTC F D	AAT N	AAG K	GGA G	ACT T N	GGT G D	1430	NC 5
445	GAG E	CAC H	GGA G E	GCT A	AGT S G	GGC G	CCC P L	AAA K	GGA G	GAA E D	AAG K	GGC G	GAT D E	ACT T	GGG G	CTG L	CCT P	GGA G	TTT F	CCA P	GGG G	TCT S	GTT V	GGC G	CCT P	AAA K	GGA G	CAC H Q	AAA K	GGA G	1520	
475	gaa E	CCT P	GGG G	GAG E	CCT P	TTA L F	ACA T	AAA K	GGT G	GAA E	aag K	GGA G	GAT D	AGA R	GGA G	GAA E	CCC P	GGC G	CTA L V	TTA L I	GGA G	CCA P S	CAG Q	GGA G	ATA I V	AAG K	GGG G	GAA E	CCT P	GGA G	1610	
505	GAT D	CCT P	GGT G	CCC P	CCT P	GGT G	TTA L	CTA L I	GGA G	AGC S	CCA P	GGG G	CTA L	AAG K	GGT G	CAG Q	CAA Q	GGA G	CCT P S	GCA A	GGT G	TCC S	ATG M	GGG G	CCC P	AGA R	GGA G	CCA P	CCA P	GGA G	1700	
535	GAT D	GTT V	GGA G	TTG L	CCA P	GGA G	GAA E	САТ Н	GGT G	ATT I	CCA P	GGA G	aaa K	CAA Q	GGC G	GTT V I	aag K	GGA G	GAG E	AAG K	GGA G	GAT D	CCG P	GGT G	GGA G	AGG R I	CTA L I	GGC G	CCT P	CCT P	1790	COL 4
565	GGA G	CTŤ L	CCA P	GGT G	CTA L P	aag K	GGT G	GAT D E	GCT A	GGG G	CCT P	CCA P	GGG G	ATA I K	AGC S	CTG L	CCA P	GGG G	AAA K E	CCA P	GGG G	TTA L	GAT D	GGA G	aat N	CCA P	GGA G	TCA S A	CCT P	GGT G	1880	
595	cca p 4	CGT R	GGG G	CCA P	aag K	GGT G	gaa E	AGA R	GGT G	CTG L	CCT P	GGG G	CTC L V	CAC H	GGT G	TCC S	CCC P	GGG G	GAC D	ACA T I	GGC G	CCA P	CCA P Q	GGT G	GTA V I	GGA G	ATC I	CCT P	GGT G	AGA R	1970	
625	ACA T	GGC G	TCC S A	CAA Q	GGA G	CCA P	GCC A	GGA G	GAG E	CCA P	GGT G	ATT I	CAG Q	GGT G	CCT P	CGA R	GGT G	CTG L	CCT P	GGA G	TTG L	CCA P	GGA G	ACT T	CCA P	GGG G	ATG M T	CCA P	GGG G	AAT N	2060	
655	GAC D	GGA G	GCT A V	CCA P	GGG G	AAG K R	GAT D	GGA G	AAG K	CCA P	GGC G	CTT L	CCA P	GGC G	CCC P	CCA P	GGT G	GAC D	CCG P	ATC I	GCA A	CTT L	CCT P	CTC L	TTG L	GGA G	GAC D	ATT I	GGC G	GCC A	2150	NC 4

Mouse $\alpha 1(XIX)$ Collagen Gene Expression

685	CTG L	CTG L	AAG K	AAC N	TTC F	TGT C	GGC G	AAC N	TGT C	CAA Q	GCC A	AAT N S	GTC V	CCT P	GGG G	CTG L	AAA K	AGC S	ATC I N	AAA K	GGC G	GAT D E	GAC D E	GGA G	AGC S G	ACT T A	GGG G	GAG E	CCT P	GGA G	2240	
715	AAG K	TAT Y	GAG E D	CCT P S	GCA A M	GCC A	AGG R	AAG K	GGT G	GAT D	GTA V I	GGG G	CCA P	CGA R	GGT G	CCT P	CCG P	GGA G	TTC F I	CCA P	GGC G	AGA R	GAA E	GGG G	CCA P	AAG K	GGA G	AGC S	aaa K	GGA G	2330	
745	GAG E	CGG R	GGC G	TAC Y	CCT P	GGA G	ATA I	CAC H P	GGG G	GAG E	AAA K	GGC G	GAT D	GAG E	GGT G	CTT L	CAA Q	GGA G	ATC I	ССТ Р	GGC G	CTC L I	TCG S P	GGA G	GCT A	CCT P	GGC G	CCA P	ACT T	GGA G	2420	COL 3
775	CCC P	CCT P	GGC G	TTA L	ACA T M	GGA G	AGA R	ACT T	GGA G	САТ Н	CCT P	GGT G	CCC P	ACT T	GGA G	GCA A	aaa K	GGT G	GAC D E	AAG K	GGT G	AGT S	GAG E D	GGA G	CCC P	CCT P	GGG G	aaa K	CCT P	GGA G	2510	
805	CCT P	CCT P	GGA G	CCC P	CCT P	GGT G	GTC V I	CCG P	CTC L F	AAT N	GAG E	GGA G R	AAC N	GGC G	ATG M	AGC S	AGT S	TTA L	TAT Y	aaa K	ATC I	CAG Q K	GGA G	GGG G	GTG V	AAT N	GTT V	ccc p	GGT G S	TAC Y	2600	NC 3
835	CCA P	GGA G	CCT P	CCG P	GGC G	CCC P	CCA P	GGT G	CCA P	AAA K	GGT G	GAT D	CCT P	GGC G	CCA P	GTG V	GGA G	GAG E	CCT P	GGT G	GCA A	ATG M	GGC G	TTG L	CCA P	GGA G	CTA L	GAA E	GGA G	TTT F	2690	
865	CCT P	GGT G	GTA V	AAG K	GGA G	GAT D	CGA R	GGC G	CCA P	GCA A	GGC G	CCC P	CCA P	GGC G	ATA I	GCC A	GGT G	ATA I M	TCG S	GGA G	AAA K	CCA P	GGT G	GCC A	CCA P	GGT G	CCT P	CCA P	GGA G	GTA V	2780	
895	CCA P	GGG G	GAG E	CAG Q P	GGT G	GAA E	AGA R	GGA G	CCT P	ATT I V	GGA G	GAT D	ACA T I	GGT G	TTT F	CCT P	GGA G	CCA P	GAG E	GGA G	CCC P	TCG S	GGG G	AAG K	CCA P	GGC G	ATA I	AAT N	GGA G	ааа К	2870	COL 2
925	GAT D	GGA G	TTA L I	CCA P	GGT G	GCT A	CAG Q	GGC G	ATC I	ATG M	GGT G	AAA K	ССТ Р	GGT G	GAC D	AGA R	GGC G	CCC P	AAA K	GGA G	GAA E	CGC R	GGT G	GAT D	CAG Q	GGA G	ATT I	CCA P	GGA G	GAC D	2960	
955	AGA R	GGC G	CCT P S	CAA Q	GGT G	GAA E	CGT R	GGG G	AAG K	сса Р	GGT G	CTT L	ACG T	GGC G	ATG M	AAG K	GGG G	GCT A	ATA I	GGC G	CCT P	GTG V M	GGA G	CCA P	GCA A P	GGA G	AGC S N	AAG K	GGC G	TCC S	3050	
985	ACT T M	GGG G	CCA P S	CCT P	GGC G	CAC H	CAA Q	GGT G	CCT P	CCA P	GGC G	AAT N S	CCT P	GGC G	ATC I	CCT P	GGC G	ACT T I	CCG P	GCT A	GAT D	GCT A	GTT V	TCA S	TTT F	GAA E	GAA E	ATA I	AAA K	CAC H K	3140	NC 2
1015	TAC Y	ATT I	AAT N	CAA Q	GAA E	GTC V	CTA L	AGG R	ATT I	TTT F	GAA E	GAG E	AGG R	atg M	GCT A	GTG V	TTT F	CTA L	TCC S	CAG Q	CTT L	ада К	CTG L	CCA P	GCA A	GCA A	ATG M	TTG L	TCT S A	GCT A	3230	NC Z
1045	CAA Q	GCT A	CAT H Y	GGG G	AGG R	CCT P	GGC G	CCA P	CCA P	GGG G	AAG K	GAC D	GGG G	TTA L	CCT P	GGA G	CCC P	CCA P	GGA G	GAT D	CCA P	GGG G	CCC P	CAA Q	GGC G	TAC Y	CGA R	GGA G	CAG Q	AAG K	3320	
1075	GGA G	GAA E	AGA R	GGT G	GAA E	CCT P	GGA G	ATT I	GGG G	CTG L	CCG P	GGG G	AGT S	CCG P	GGT G	CTT L	CCT P	GGA G	TCT S T	TCA S	GCA A	GTG V L	GGT G	TTG L	CCG P	GGC G	TCA S	CCA P	GGT G	GCC A	3410	COL 1
1105	CCG P	GGG G	CCT P	CAA Q	GGA G	CCC P	CCG P	GGA G	CCC P	AGT S	GGA G	AGA R		AAT N	CCA P	GAA E	GAT D		CTC L	TAT Y	CCT P	GCA A V	CCT P S	ССТ Р Н	CCC P A	CAC H	CAG Q	CAG Q R	GCA A T	GGC G	3500	NC 1
1135	GGG G	AAG K N	TGA * *		3																										3509	
	AAAA TCTC GAG GGT TGC CTA	AGACO GATAO FTCT/ FTTG/ FGTTO FGTG/	GAGA CAGA ACGGO ATTCO GACTO	GAAGA AGCAC CTTCC CTGAA GTCT IGTT	ACATO GACAT GGAAG ATTTT FAGG/ FATC/	GCG1 CCA AAA AAA AAAC1 ACTG2 AGAA	ICTAC AGC/ ATGC ITTTC ATTTI	TGC: ACCA CAAGO TAAC TAACA	PATCI ATCIG CGAGC PTTAI AAGAA	CCAC CTGT CTGT CTTC	GCCA CTAT CATC GATTA CTATA	ACTTO TCTO ACTTT ACTGI	GAGCO GACAO ICTTO ICTTO ICCT7	GCCI STAAC CAGAI CATAT	ACTGO GAGCI AAAAI ITTAI ICAT	CAGGC AGATC ATCAC ATCAC ATTI CGAI	CGTG CGTG CGTG CGTG CGTG CTTA CTTA	ACAAC ATTTT AGACC ATCG'I IGCAC	CACO CAG SATT TAC SAAA	GTGCT ATGTT IGGT7 ITTAC AAAAT	PC TC PTGCO AC AC STTT PA TAO TGAO	AAGGO CAAC AGACO TTCT CAATI	CTTAT FTCTT GACAC FTCTC NAGAC	IGAGI ITCCA CAGAC GCTCI GATIX CATISC	TTTT ATATO CCTTO FTTTO CGCT C	IGGAC STTCT STGAZ SAGAT	CTGCI IGTTI ACTCO ITGA# ACTTI	TTAT TCAT TCA GTA AGTAC	rttt NGTGX NCGTG STAC# NAAAG	AGTTT GCTCT GACTA ATTAC CCAAA	3629 3749 3869 3989 4109 4205	

FIG. 2—continued

weeks after the first injection. The titers of antisera were measured with enzyme-linked immunosorbent assay (13).

Collagen Purification—All steps were carried out at 4 °C or on ice (14). Brains, dissected from 40 5-week-old mice, were homogenized in 500 ml of cold isotonic buffer (0.25 M sucrose, 10 mM HEPES, pH 7.0, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM N-ethylmale-imide), and then stirred for 60 min. The homogenate was centrifuged for

30 min at 15,000 rpm. The pellet was suspended in 500 ml of extraction buffer (1 M NaCl, 50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM *N*-ethylmaleimide) and incubated for 48 h with stirring. The suspension was centrifuged at 35,000 rpm for 30 min, and the solubilized proteins were precipitated with 4 M NaCl of final concentration. The precipitate was collected by centrifugation at 25,000 rpm for 30 min, and then the pellet was suspended in 0.5 M acetic

FIG. 2. Nucleotide sequence of cDNA (*top*) and deduced amino acid sequence (*middle*) of the mouse $\alpha 1$ (XIX) collagen chain. The amino acid sequence of the human (*bottom*) has been aligned with that of the mouse chain. The residues of the human differing from those of the mouse only are shown. The collagenous domains (*COL*) are indicated by *boxes*, and those of the imperfections are indicated by *small boxes* in them. The *asterisks*, the *triangle*, and *open squares* indicate the amino acid residues missing in the mouse and a stop codon, the putative signal peptide cleavage site, and cysteine residues. The *horizontal arrows* show the positions of the primers; primers 1–4 were used for generating cDNA clone, ME PCR, primer 5 and 6 for RT-PCR analysis, and primer 7 and 8 for generating DNA fragment to be ligated to expression vector.

TABLE I Developmental expression of $\alpha 1(XIX)$ collagen mRNAs determined by RT-PCR analysis

The symbols used are: ++, strongly positive; +, weakly positive; $\pm,$ trace; –, negative; and ND, not determined.

	18 d.p.c. embryo	5-Week-old adult
Muscle	+	_a
Heart	+	_
Aorta	ND	<u>+</u>
Skin	+	_a
Intestine	+	_
Brain		
Whole	+	
Cerebrum		++
Cerebellum		+
Eye	ND	+
Limb	+	ND
Vertebrae	+	ND
Tail	+	_
Joint	ND	_
Sternum	ND	_
Calvaria	+	ND
Lung	+	<u>+</u>
Kidney	+	<u>+</u>
Liver	_	_
Spleen	ND	_
Thyroid	ND	_
Adrenal	ND	_
Testis	ND	+

^{*a*} The transcripts are usually not detectable in this condition. Compare to the results of competitive RT-PCR in Table III.

TABLE II Expression of $\alpha 1(XIX)$ collagen mRNAs in the cultured cells determined by RT-PCR analysis

The symbols used are: ++, strongly positive; +, weakly positive; \pm , trace; and -, negative.

Rhabdomyosarcoma (human)	
RD	++
A204	_
KYM1	_
Glioma (human)	
U251MG	++
U373MG	—
A172	-
Neuroblastoma (human)	
TGW	<u>+</u>
NB1	-
Fibroblast (human)	
Infant	+
Adult	—
Fibroblast (mouse)	
Embryo	+
Adult	<u>+</u>
Lymphocyte (human)	
EB virus transformed	—

acid. The insoluble material was removed by centrifugation at 35,000 rpm for 30 min. The supernatant was dialyzed against 5 mM acetic acid and lyophilized. The sample was resolved with 200 μ l of 50 mM acetic acid and stored at 4 °C.

Western Blotting—Proteins were separated on 6.0% SDS-PAGE in the presence or absence of 2-mercaptoethanol (12). An aliquot of the sample was also digested with bacterial collagenase before loading onto the gel. The sample for digestion was first neutralized with 0.5 M NaOH and then incubated with bacterial collagenase solution (250 units/ml Wakojunyaku Form III; 50 mM Tris-HCl, pH 7.5, 0.2 M NaCl, 5 mM CaCl₂) containing 5.5 mM CaCl₂ for 5 h at 37 °C. Samples were transferred onto polyvinyliden difluoride membranes electrophoretically (15), and the gel was stained with Coomassie Brilliant Blue R. The transferred sample was allowed to react with anti- α 1(XIX)COL antibodies, followed by incubation with the peroxidase-conjugated secondary antibodies against anti-rabbit IgG. The signals were detected by enhanced chemiluminescence Western blotting detection reagents (Amersham Corp.).



FIG. 3. Competitive PCR products run on the agarose gels. The gel shown here contains RT-PCR products of one representative experiment using the muscle, skin, brain, and liver RNAs as templates. The concentration of the gels is 2.0% for $\alpha 1(XIX)$ and 2.5% for β actin. The products from RNA of β actin are shown only from the experiment using the skin. The panels on the left show the products from RNAs of 18 d.p.c. mouse embryo, and on the right from 5-week-adult mouse. The RT-PCR products of the competitor (open arrowhead) has been designed to be 51 bp (XIX collagen) and 61 bp (β actin) smaller than those of the endogenous product (closed arrowhead). On each gel, lanes left to right contain amplified DNA synthesized from a constant amount of tissue cDNA and decreasing amounts of competitor DNAs. The DNA competitor in the reaction mix was as follows: $\alpha 1(XIX)$ collagen competitor: lane A, 1 pg; lane B, 500 fg; lane C, 250 fg; lane D, 125 fg; lane E, 62.5 fg; lane F, 31.3 fg; lane G, 15.6 fg; lane H, 7.8 fg; lane I, 3.9 fg; lane J, 1.9 fg; and lane K, 1 fg; and β actin competitor: lane 1, 50 pg; lane 2, 25 pg; lane 3, 12.5 pg; lane 4, 6.3 pg; lane 5, 3.2 pg; and lane 6, 1.6 pg.

RESULTS AND DISCUSSION

Primary Structure of Mouse $\alpha 1(XIX)$ Chain—To identify the best source for $\alpha 1(XIX)$ collagen, we initially performed RT-PCR amplifications using RNA from a variety of embryonic and adult mouse tissues and a couple of sets of primers derived from the human sequence. The most convincing results of this initial survey were obtained using embryonic tissues. Sequencing of these amplification products showed that the clones were the mouse counterparts of the human COL19A1 gene. Based on these data, we constructed and screened a cDNA library from 18 d.p.c. mouse whole embryo. Five overlapping clones were isolated and, upon sequencing, found to cover all but 0.6 kb of the region coding for the mouse $\alpha 1(XIX)$ chain; this last gap was finally covered by RT-PCR amplification (Fig. 1).

The deduced amino acid sequence of the mouse $\alpha 1(XIX)$ chain shows 82% identity to the human counterpart with the highest level of sequence conservation at the carboxyl termini than the amino termini. All of the potentially important structural-functional features previously noted in the human $\alpha 1(XIX)$ chain are also conserved in the mouse polypeptide (Fig. 2) The major difference between the two mammalian polypeptides is the total number of 1,136 amino acids in mouse *versus* 1,142 amino acids in human. The differences are due to the deletion of three amino acids in each NC6 and NC5 domain and the substitution of cysteinyl residues for serinyl residues in the signal peptide of the mouse chain. Additional structural differences include the NC5 and NC3 domains, which are shorter in the mouse compared with the human chain, and the relative position of small imperfection in the COL5 domain. These few differences notwithstanding, the primary structure of the $\alpha 1(XIX)$ collagen chains is remarkably conserved between the two mammalian species.

Tissue Distribution of Col19a1-To define the pattern of expression of Col19a1,² we surveyed tissues from various embryonic stages and 5-week-old mouse tissues. The transcripts were readily detectable by RT-PCR analysis in the whole embryo at 11, 12, and 14 d.p.c. (data not shown). Positive tissues of 18 d.p.c. embryo include limbs, vertebrae, heart, brain, tail, kidneys, calvaria, lung, muscle, skin, and intestine (Table I). A very different pattern was observed in adult tissues where Col19a1 expression was only seen in the cerebrum, cerebellum, eyes, and testis, and perhaps in the aorta, lungs, and kidneys (Table I). Based on these data, we used the same approach to identify cultured cells that accumulate COL19A1 (and Col19a1) transcripts. This second survey included human rhabdomyosarcoma cell lines (RD, A204, and KYM1), human glioma cell lines (U251MG, U373MG, and A172), human neuroblastoma cell lines (TGW and NB1), human normal skin fibroblasts (infant and adult), and mouse normal skin fibroblasts (18 d.p.c. embryo and adult) (Table II). Among malignant cell lines, only RD (rhabdomyosarcoma) and U251MG (glioma) showed significant COL19A1 mRNA accumulation. Detectable amounts were also noted in human neonatal fibroblasts, mouse embryo fibroblasts, and to a much lesser extent, in adult fibroblasts.

To examine whether *Col19a1* gene expression may change during development, we compared transcript levels in the muscle, skin, brain, and liver of 18 d.p.c. embryos and 5-week-old mice using the quantitative technique of competitive RT-PCR (Fig. 3). Relative quantitation of amplification from the endogenous transcript and the competitor plasmid revealed that 1 μ l of cDNA from muscle, skin, and brain of 18 d.p.c. embryo and 5-week-old mouse contained 52.3, 7.1, 24.5, 0.7, 0.4, and 149.3 fg, respectively. When normalized to the β actin values, the relative amounts of *Col19a1* transcripts were significantly higher in embryonic muscle and skin than in the adult counterparts, whereas transcript levels in the brain were approximately 10 times more in adult animals than embryos (Table III).

To identify the protein in a tissue that expresses the $\alpha 1(XIX)$ mRNA, we utilized specific polyclonal antibodies (Fig. 4) in a Western blotting analysis of mouse adult brain tissue. The

 2 The symbol Col19a1 for the mouse $\alpha 1(\rm XIX)$ collagen gene is approved HGMW.

antibodies were raised in rabbits against an $\alpha 1(XIX)$ recombinant peptide. As shown Fig. 5*C*, the antibodies reacted with peptides of 150 and 145 kDa in size from a fraction of neutral salt extraction of mouse adult brain. Consistent with their identity, the 150- and 145-kDa bands were digested with bacterial collagenase (Fig. 5*C*, *lane 2*). Under nonreducing conditions, the reacting peptide migrated at about 400 kDa which probably represents the trimeric form of type XIX collagen.

The above results suggest that type XIX collagen is an embryonic FACIT, since its expression is mostly confined to embryonic tissues and only a few adult organ systems. High Col19a1 expression in a couple of tumor lines indirectly supports the idea of a mostly embryonic gene product. We postulate that the uniqueness of this FACIT molecule extends to the kind of supermolecular aggregate it is associated with. We note that Col19a1 expression in the embryo does not follow the

TABLE III Mean ratios of mouse $\alpha 1(XIX)$ collagen to β actin mRNA (×10⁻³) Values are means ± S.D. n = 5 (p < 0.015). ND, not detectable.

	18 d.p.c. embryo	5-Week adult
Muscle Skin Brain Liver	$\begin{array}{c} 8.03 \pm 4.13 \\ 2.00 \pm 1.20 \\ 1.15 \pm 0.47 \\ \mathrm{ND} \end{array}$	$0.14 \pm 0.10 \\ 0.15 \pm 0.19 \\ 13.57 \pm 8.08 \\ \mathrm{ND}$



FIG. 4. Enzyme-linked immunosorbent assay using anti-type XIX collagen polyclonal antibody. The antibody reacted with the $\alpha 1$ (XIX)-MBP fusion protein (*closed circles*) but never cross-reacted with total *Escherichia coli* proteins containing MBP (*open squares*).



FIG. 5. Protein analysis for the type XIX collagen using mouse adult brain extract. A, purified recombinant proteins were run on 12.5% SDS-PAGE gel under a reducing condition and stained with Coomassie Brilliant Blue. Lane 1, MBP-COL fusion protein purified with amylose affinity column; *lane* 2, digested fusion protein with factor Xa; *lane* 3, digested protein with bacterial collagenase. An arrow indicates COL peptide in *lane* 2, which disappears in *lane* 3. B, gel electrophoresis of the extract prepared from mouse brain. The sample was separated on 6.0% SDS-PAGE gel and stained with Coomassie Brilliant Blue. *Lane* 1, neutral 1 M NaCl extract from mouse adult brain tissues with β-mercapto-ethanol; *lane* 2, the same sample of *lane* 1 treated with bacterial collagenase; *lane* 3, the same sample of *lane* 1 mouse type I collagen digested with bacterial collagenase. C, Western blot analysis for neutral 1 M NaCl extract from mouse adult brain tissues. *Lanes* 1–3 correspond with *lanes* 1–3 of B, respectively. Molecular mass is indicated on the *left* side of the panels.



FIG. 6. Northern blot analysis of the $\alpha 1$ (XIX) mRNA. A, the mRNA samples isolated from human rhabdomyosarcoma cells RD (3 μ g, lanes 1 and 2) and 18 d.p.c. mouse whole embryo (5 µg, lanes 3 and 4) were electrophoresed on 0.8% agarose gels, blotted onto nylon filters, and hybridized with various probes. Each lane was hybridized with 5' EcoRI/PstI fragment of HY 67 that encodes a portion of NC 6 domain (4) (5' human probe (lane 1)), 3' HindIII fragment of KI 65 that encodes a portion of 3'-untranslated region (4) (3' human probe (lane 2)), 5' EcoRI fragment of ME12 that encodes 5'-untranslated region and a portion of NC 6 domain (indicated by (1) in Fig. 1) (5' mouse probe (lane 3)), and 3' EcoRI/PstI fragment of ME14 that encodes a portion of 3'-untranslated region (indicated by (2) in Fig. 1) (3' mouse probe (lane 4)). B, the mRNA samples isolated from RD (3 µg, lane 1) was hybridized with 5' human probe, and those from mouse 18 d.p.c. whole embryo (5 µg, lane 2) and adult brain (5 µg, lane 3) with 5' mouse probe. C, the mRNA samples isolated from RD (3 µg, lane 1), U251MG (human glioma, 5 µg, lane 2), human infant fibroblasts (5 μ g, lane 3), and human adult fibroblasts (5 μ g, lane 4) were hybridized with 5' human probe.

pattern of either of the major fibrillar collagens (types I or II) for it includes both cartilagenous and non-cartilagenous tissues. Even more intriguing is the expression of type XIX in the adult aorta, testis, and brain. The last organ, in particular, raises provoking new ideas. For example, type XIX collagen may be produced by glial cells to serve as a connecting bridge of the proteoglycan network or as an anchor for the surrounding cells. Irrespective of the alternatives, the evidence strongly supports the original prediction of Gordon and Olsen (16) for the existence of FACIT molecules that may provide highly specific properties to selected organ systems and/or at specific developmental stages.

Alternative Splicing of the COL19A1 Transcript—We have previously reported an unusual number of alternatively spliced COL19A1 products in the rhabdomyosarcoma cell line RD (4). Here we examined whether or not alternatively spliced products appear to be tissue- and/or stage-specific. Northern analysis of mRNA from 18 d.p.c. whole embryo yielded a single band with 5' (Fig. 6A, lane 3) and 3' (lane 4) cDNA fragment as probes. Transcripts from mouse adult brain also showed a single band with more strong signal (Fig. 6B, lane 3) than that from 18 d.p.c. mouse embryo (Fig. 6B, lane 2) when 5' probe was used. Likewise, mRNA from infant skin fibroblasts showed only one hybridizing species (Fig. 6C, lane 3). In contrast, mRNA from glioma cell line (U251MG) yielded multiple bands (Fig. 6C, lane 2).

Alternative splicing is a widely used means to generate protein isoforms or even functionally distinct products from the same transcript. Alternative splicing has been reported to occur for several different collagen types (17–24), although its functional significance is understood only for a few of them. In the case of the $\alpha 1(XIX)$ chain, the results are apparently contradictory. Unlike two tumor lines, multiple transcripts have not been seen in the present survey of normal tissues and cultured cells. We therefore conclude that the alternatively

spliced COL19A1 transcripts seen in the tumor cells are probably aberrant products of no physiological significance and without counterparts in the normal organism. It is however unclear the reason for and the mechanism behind this strange phenomenon in cancer cells.

Conclusion-In summary, this study has provided new and important information about the possible role of type XIX collagen. We believe the expression data are consistent with the idea that type XIX collagen is somehow involved in the assembly of specialized structures of the developing embryo and/or the function of particular organ systems in the adult organism. Irrespective of the hypothesis, it is safe to predict that type XIX collagen will eventually prove to be unique among other FACIT members. The information and reagents produced by the present study provide the means to address this question using the powerful technique of gene targeting in the mouse.

Acknowledgments-We are indebted to Dr. F. Ramirez for the review and critical comments of the manuscript. We also thank M. Kohmoto and K. Frith for typing the manuscript.

REFERENCES

- 1. van der Rest, M., and Garrone, R. (1991) FASEB J. 5, 2814-2823
- 2. Kivirikko, K. I. (1993) Ann. Med. 25, 113-126
- Myers, J. C., Yang, H., D'Ippolito, J. A., Presente, A., Miller, M. K., and Dion, 3. A. S. (1994) J. Biol. Chem. 269, 18549–18557
 Inoguchi, K., Yoshioka, H., Khaleduzzaman, M., and Ninomiya, Y. (1995)
- J. Biochem. (Tokyo) 117, 137-146
- 5. Shaw, L. M., and Olsen, B. R. (1991) Trends Biochem. Sci. 16, 191-194
- Diab, M., Wu, J. J., and Eyre, D. R. (1996) Biochem. J. 314, 327-332 7. Yoshioka, H., Zang, H., Ramirez, F., Mattei, M.-G., Moradi-Ameli, M., van der
- Rest, M., and Gordon, M. K. (1992) Genomics 13, 884-886 8. Myers, J. C., Sun, M. J., D'Ippolito, J. A., Jabs, E. W., Neilson, E. G., and Dion,
- A. S. (1993) Gene (Amst.) 123, 211-217 9. Oh, S. P., Taylor, R. W., Gerecke, D. R., Rochelle, J. M., Seldin, M. F., and Olsen, B. R. (1992) Genomics 14, 225-231
- 10. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 11. Wang, A. M., Doyle, M. V., and Mark, D. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9717-9721

8626-8633

- Laemmli, U. K. (1970) Nature 227, 680-685
 Rennard, S. I., Berg, R., Martin, G. R., Foidart, J. M., and Robey, G. P. (1980) Anal. Biochem. 104, 205-214
 Dublet, B., Oh, S., Sugrue, S. P., Gordon, M. K., Gerecke, D. R., Olsen, B. R., and van der Rest, M. (1989) J. Biol. Chem. 264, 13150-13156
 Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350, 4354
- 76, 4350 4354
- Gordon, M. K., and Olsen, B. R. (1990) Curr. Opin. Cell Biol. 2, 833–838
 Sandell, L. J., Morris, N., Robbins, J. R., and Goldring, M. B. (1991) J. Cell Biol. 114, 1307–1319
- 18. Strokes, D. G., Saitta, B., Timpl, R., and Chu, M.-L. (1991) J. Biol. Chem. 266,
- 19. Oxford, J. T., Doege, K. J., and Morris, N. P. (1995) J. Biol. Chem. 270, 9478-9485
- 20. Zhidkova, N. I., Justice, S. K., and Mayne, R. (1995) J. Biol. Chem. 270, 9486-9493
- Yoshioka, H., Inoguchi, K., Khaleduzzaman, M., Ninomiya, Y., Andrikopoulos, K., and Ramirez, F. (1995) *Genomics* 28, 337–340
 Tsumaki, N., and Kimura, T. (1995) *J. Biol. Chem.* 270, 2372–2378
 Trueb, J., and Trueb, B. (1992) *Biochem. Biophys. Acta* 1171, 97–98
 Tikka, L., Pihlajaniemi, T., Henttu, P., Prockop, D. J., and Tryggavason, K. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 7491–7495