ARTICLE
Journal of Cellular Biochemistry 112:541–547 (2011)

Journal of Cellular Biochemistry

The Procollagen Type III, Alpha 1 (COL3A1) Gene First Intron Expresses Poly-A$^+$ RNA Corresponding to Multiple ESTs and Putative miRNAs

Kenneth M. Sterling*

Whitney Laboratory for Marine Bioscience, University of Florida, 9505 Ocean Shore Boulevard, St. Augustine, Florida 32080-8610

ABSTRACT

The mouse COL3A1 first intron is 9684 bp. RNA's of approximately 1.6 and 3.0 kb were detected by Northern hybridization analysis of poly-A RNA from fetal mice and total RNA from suckling and adult mouse intestine using ^{32}P-labeled, anti-sense RNA synthesized from a mouse COL3A1 first intron, 5 prime region, 5.4 kb *Xba* I fragment (1655–7030 bp), recombinant plasmid (pPI5.4x). Expression of the 1.6 and 3.0 kb RNA's was significantly reduced in adult mouse intestine, indicating that these RNAs are developmentally regulated. "BLAST" analysis indicated that the mouse first intron 5 prime sequence has 94–100% identity to 13 mouse ESTs. These mouse first intron EST's lie within the 5.4 *Xba* I fragment of the mouse COL3A1 first intron. Two of the mouse first intron EST's have significant identity to known miRNA, mature sequences, mmu-miR-466f-3P, mmu-miR-1187, and mmu-miR-574-5P as well as others. Predicted targets for mmu-miR-466f-3P include COL1A1, COL19A1, COL11A2, COL4A1, and COL4A5 indicating that COL3A1 intronic miRNAs may regulate the expression of other collagen genes in development. J. Cell. Biochem. 112: 541–547, 2011. © 2010 Wiley-Liss, Inc.

KEY WORDS: COLLAGEN; INTRON; miRNA

An analysis of mRNA abundance in developing rat intestine using a ^{32}P-labeled first intron-second exon procollagen type III (COL3A1) genomic DNA fragment, pMCS-1 [Liau et al., 1985b] indicated the presence of the mature COL3A1 mRNA of 5.4 kb and two additional RNAs of 1.6 and 2.9 kb [Walsh et al., 1987]. The two smaller RNAs were believed to be derived from the rat COL3A1 first intron. This observation led to the present study.

The collagens are a family of ancient structural proteins that contains over 40 separate genes coding for precursor chains that form at least 28 different mature triple-helical molecules [Kielty and Grant, 2002; Chan et al., 2008]. Mutations in collagen genes give rise to multiple disease states affecting the tissues in which the genes are expressed, for example, COL3A1, Ehlers-Danlos syndrome type IV [Kuivaniemi et al., 1997; Byers, 2000]. COL3A1 is a fibrillar collagen that consists of three alpha 1 peptide chains forming a triple-helix and normally co-expressed in tissues with the more abundant COL1A1 and COL1A2 genes that form Type I fibrillar collagen [Chan et al., 2008].

COL3A1 expression is altered in disease states such as cancer [Turashvili1 et al., 2007], fibrosis [Gant et al., 2003], viral infection [Abend et al., 2010], and inflammation [Wu and Chakravarti, 2007]. COL3A1 expression is regulated by DNA binding transcriptional factors and TGF-beta [Oleggini et al., 2000; Verrecchia et al., 2001; Verrecchia and Mauviel, 2007]. COL3A1 expression is also altered in developing intestine by glucocorticoids [Walsh et al., 1987].

In an effort to identify the nature of the rat COL3A1 intronic RNAs previously observed [Walsh et al., 1987] and the mouse first intron RNAs reported here, bioinformatics analysis was carried out using the mouse and rat COL3A1 intron 1 sequences resulting in the identification ESTs with up to 100% sequence identity to the intron sequences. Expressed sequence tags, EST's, are cDNA libraries generated from tissue-specific mRNAs of which on average 200–300 bp have been sequenced [Adams et al., 1991]. Bioinformatics analysis using the human COL3A1 first intron also resulted in the identification of multiple ESTs. Similar analysis with the chicken COL3A1 first intron resulted in the identification of only one EST containing several putative miRNAs.

MicroRNAs (miRNAs) are a class of small noncoding RNAs that have a significant regulatory role in multicellular organisms and are also expressed by viruses in infected cells [Cummins and Velculescu, 2006; Boss et al., 2009]. miRNAs are essential regulators of diverse biological processes, including cell division, apoptosis, and

Abbreviations used: EST, expressed sequence tag, miRNA, MicroRNA.

*Correspondence to: Dr. Kenneth M. Sterling, 9505 Ocean Shore Boulevard, St. Augustine, FL 32080-8610.
E-mail: ksterling@whitney.ufl.edu

Received 25 October 2010; Accepted 27 October 2010 • DOI 10.1002/jcb.22944 • © 2010 Wiley-Liss, Inc.
Published online 22 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

metabolism [Bartel, 2004; Kloosterman and Plasterk, 2006]. miRNA precursors are processed sequentially by the enzymes Drosha and Dicer to yield mature 22-nt long single-stranded miRNAs [Ambros et al., 2003; Pillai et al., 2007]. miRNAs are believed to influence gene expression at the level of translation of target mRNAs, but may have other mechanisms of action [Garzon et al., 2006; Kloosterman and Plasterk, 2006].

The miRBase [Griffiths-Jones et al., 2008] was screened for mature miRNAs using the mouse, rat, human and chicken COL3A1, intron 1 ESTs. The mouse, rat and human COL3A1, intron 1 sequences corresponding to several ESTs have significant identity to known miRNAs while the chicken COL3A1 first intron EST has identity to several known miRNAs. The mouse, rat, and human intron miRNA sequences also coincide with inverted repeats [Rice et al., 2000]. These putative miRNAs are examples of intron-derived miRNAs [Lin et al., 2006]. The mouse, rat, human, and chicken miRNAs have multiple predicted targets [Griffiths-Jones et al., 2006].

MATERIALS AND METHODS

PLASMID

The 5.4 kb, first intron, *Xba* I fragment from lambda-PMC3A-5 [Liau et al., 1985a] that contains the mouse COL3A1 5′-flanking, first exon, first intron and second exon sequences (Fig. 1) was subcloned into pGEM4Z (Promega, Madison, WI). The resulting recombinant, mouse COL3A1 first intron containing plasmid, pPI5.4x, was sequenced by the dideoxy-chain termination method of Sanger et al. [1977] using the Sequenase sequencing kit (USB, Cleveland, OH), [alpha-^{32}P]dATP and SP6 and T7 promoter primers.

ISOTOPES

[Alpha-^{32}P]UTP and [alpha-^{32}P]dATP was purchased from Dupont-NEN (Boston, MA).

RNA LABELING

pPI5.4 was linearized with *Eco*RI and agarose gel purified. Radiolabeled antisense RNA was obtained by incorporation of [alpha-^{32}P]UTP into nascent chains using T7 DNA polymerases (Promega).

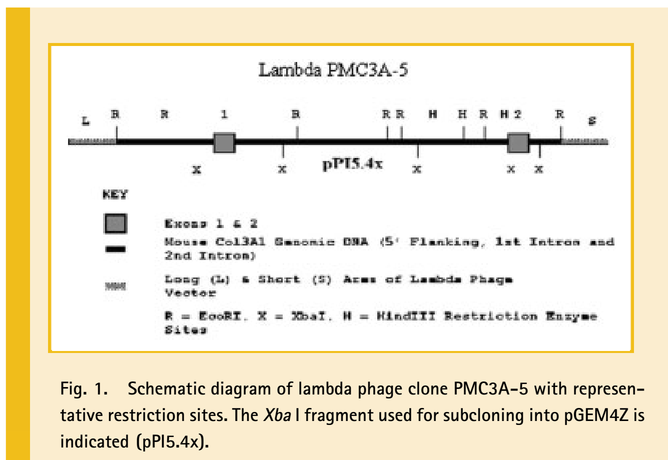

Fig. 1. Schematic diagram of lambda phage clone PMC3A-5 with representative restriction sites. The *Xba* I fragment used for subcloning into pGEM4Z is indicated (pPI5.4x).

RNA ISOLATION

Total RNA from fetal, neonatal, and adult mice was prepared by the guanidinium–phenol–chloroform procedure of Chomczynski and Sacchi [1987]. Tissues were washed in ice-cold PBS and homogenized in 4 M guanidine isothiocyanate, 25 mM Na_3 citrate, pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercapto-ethanol. The homogenate was mixed vigorously with 2 M NaOAc, pH 4, phenol and chloroform–isoamyl alcohol and incubated on ice for 15 min. The mixture was centrifuged and subsequently precipitated with isopropanol. The pellet was re-extracted with the guanidine isothiocyanate solution, reprecipitated with isopropanol, washed with ethanol and redissolved in sterile RNAase free H_2O. All glassware and appropriate solutions were treated with diethylpyrocarbonate and autoclaved to minimize RNase contamination. Purity and amount of RNA was determined by A_{260}/A_{280} measurements. Poly-A^+ RNA was purified by oligo-dt chromatography.

SEPARATION OF RNA BY AGAROSE GEL ELECTROPHORESIS

Two micrograms of poly-A^+ and poly-A^- RNA or 20 μg of total RNA were separated in 1.4% agarose-formaldehyde gels and transferred to BA85 nitrocellulose paper (Schleicher and Scheull) as described by Maniatais et al. [1982].

NORTHERN BLOT HYBRIDIZATION

The RNA filters were prehybridized for 30 min at 60°C in 50% formamide, 6X SSC, 1.0% SDS, 0.1% Tween 20, and 100 μg/ml tRNA. Hybridization was carried out for 12–18 h with the addition of 1×10^6 cpm of labeled RNA probe. Washing was done twice in 1X SSC. 0.1% SDS at 22°C for 30 min followed by washing twice in 0.1X SSC, 0.1% SDS at 65°C for 30 min.

BASIC ALIGNMENT SEARCH TOOL (BLAST) ANALYSIS

The mouse [Toman and de Crombrugghe, 1994], rat [Rat Genome Sequencing Project Consortium, 2004], human [Hillier et al., 2005], and chicken [International Chicken Genome Sequencing Consortium, 2004] COL3A1 first intron sequences were used to BLAST The Genbank, EST, and nucleotide data banks [Altschul et al., 1997].

MicroRNA BLAST

The miRBase Sequence Database [Griffiths-Jones et al., 2006, 2008] was searched using mouse, rat, human, and chicken COL3A1 first intron and EST sequences.

MicroRNA TARGET SCAN

The miRBase Target Database [Griffiths-Jones et al., 2006, 2007] was searched for predicted target genes of known miRNA sequences identified within the mouse, rat, human, and chicken COL3A1 first intron sequences.

PROMOTER PREDICTION

Potential intronic promoters within the COL3A1 first intron sequences were identified by Promoter Scan, PROSCAN Version 1.7 suite of programs [Pedersen et al., 1999; Bajic et al., 2004].

EXON/INTRON PREDICTION

Prediction of intron/exon junctions of the COL3A1 first intron sequences was done with GENSCAN [Burge and Karlin, 1997].

INVERTED REPEAT IDENTIFICATION

Inverted repeats within the COL3A1 first intron sequences were identified using inverted software [Rice et al., 2000].

RESULTS

Northern blot analysis of adult and 12-day-old suckling mice intestinal total RNA indicated the presence of two transcripts of approximately 1.6 and 3.0 kb (Fig. 2) using a ^{32}P-labeled antisense RNA probe synthesized from the plasmid pPI5.4x (Fig. 1). Northern blot analysis also indicated the presence of a 3.0 kb transcript from fetal mouse total poly-A^{+} RNA (Fig. 3).

Blast analysis of the mouse COL3A1 intron 1 sequence resulted in the identification of 13 mouse ESTs consisting of three overlapping sets of forward strand transcripts (BX335218, BB664446: CJ160307, CN668922, BB648452: BB534949, BG088384, AA543153, BY671278, DV075019, BB363623) and one reverse strand set of transcripts (AW557338, BG075843) (Fig. 4A). Blast analysis of the rat COL3A1 intron 1 sequence identified nine mouse ESTs with significant sequence identity all of which were the same as those identified for the mouse COL3A1 intron 1 (Fig. 4B).

A similar analysis of the human COL3A1 intron 1 revealed identity to 29 human ESTs consisting of two sets of overlapping forward strand transcripts and a single forward strand transcript (BG900720, DB230813, AL709028, AL701285, BX496725, BX955745, BX955750, BX472623: AW895760, DA572628, AU117559, BX473342, AF148885, BI494551, CD660705, and BF329169, respectively) and three sets of reverse strand transcripts

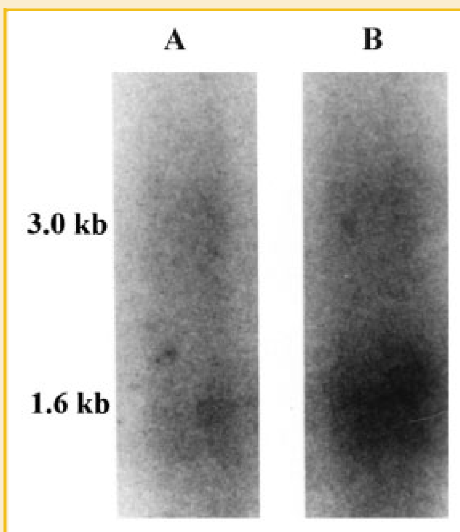

Fig. 2. A: Adult mouse intestine total RNA probed with ^{32}P-labeled antisense RNA synthesized from pPI5.4x. B: Suckling mouse intestine total RNA probed with ^{32}P-labeled antisense RNA synthesized from pPI5.4x.

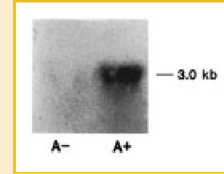

Fig. 3. Fetal mouse poly-A^{-} RNA probed with ^{32}P-labeled antisense RNA synthesized from pPI5.4x. Fetal mouse poly-A^{+} RNA probed with ^{32}P-labeled antisense RNA synthesized from pPI5.4x.

(DR980791: AW839433 and BQ008705, BQ025671, AA989181, AI827248, AU144355, AU146808, AW058627, BQ007128) (Fig. 4C). A further search analysis revealed two noncoding RNA transcripts of 1.7 kb (AK021531) and 4.4 kb (BX649097) derived from the human COL3A1 first intron and a 628 bp alternately spliced transcript (CN483491.1) derived from the human COL3A1 exon 1, intron 1, exon 2, intron 2, and exon 3 sequences (Fig. 4C).

Blast analysis of the chicken COL3A1 intron 1 identified one intron-derived EST (BU442634) (Fig. 4D). There are also forward and reverse strand predicted promoters in the mouse COL3A1 first intron

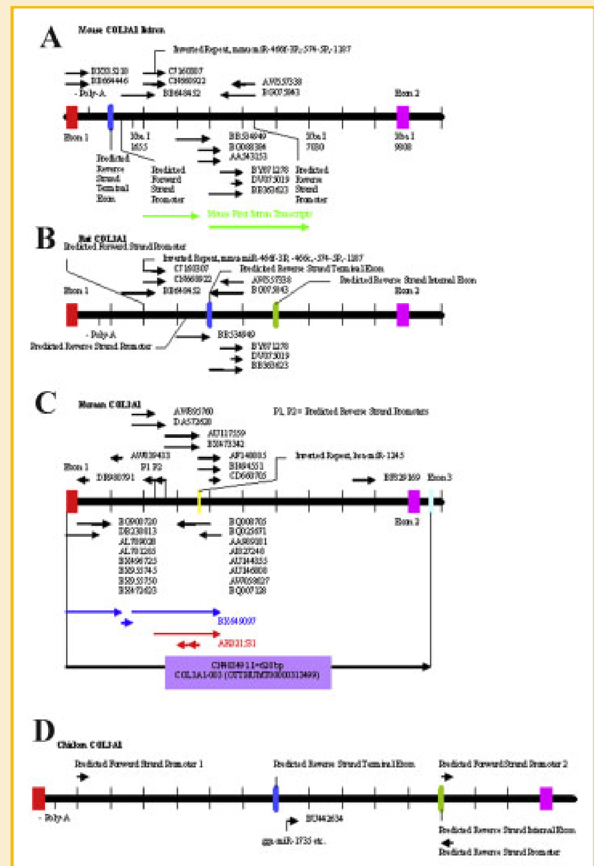

Fig. 4. Schematic diagrams of the mouse (A), rat (B), human (C), and chicken (D) COL3A1 first introns. The locations of intronic transcripts (tentative for the mouse), ESTs, inverted repeats, predicted miRNAs, promoters, exons and poly-A sites are shown.

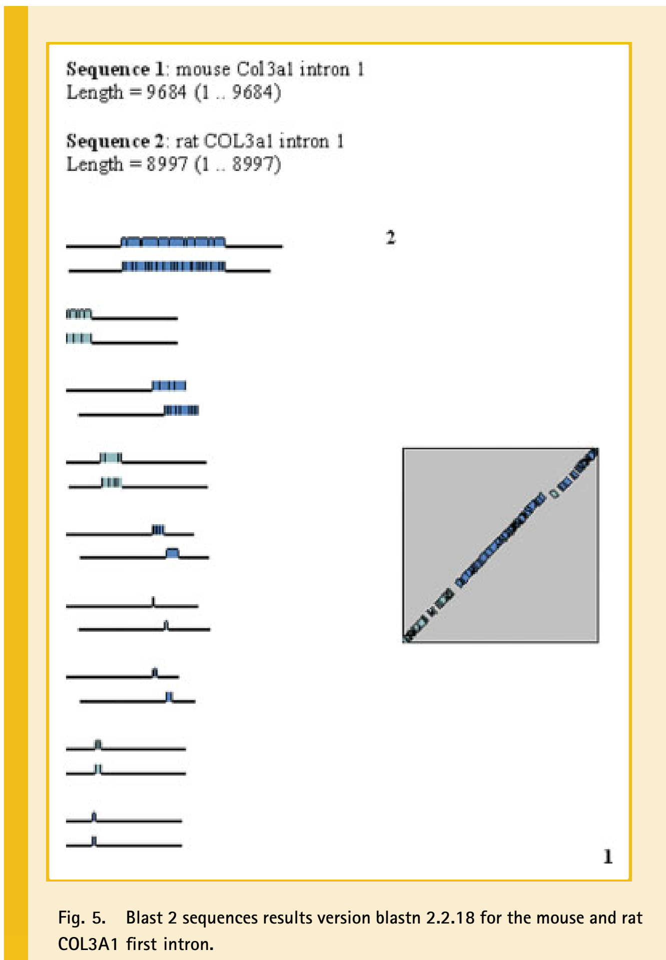

Fig. 5. Blast 2 sequences results version blastn 2.2.18 for the mouse and rat COL3A1 first intron.

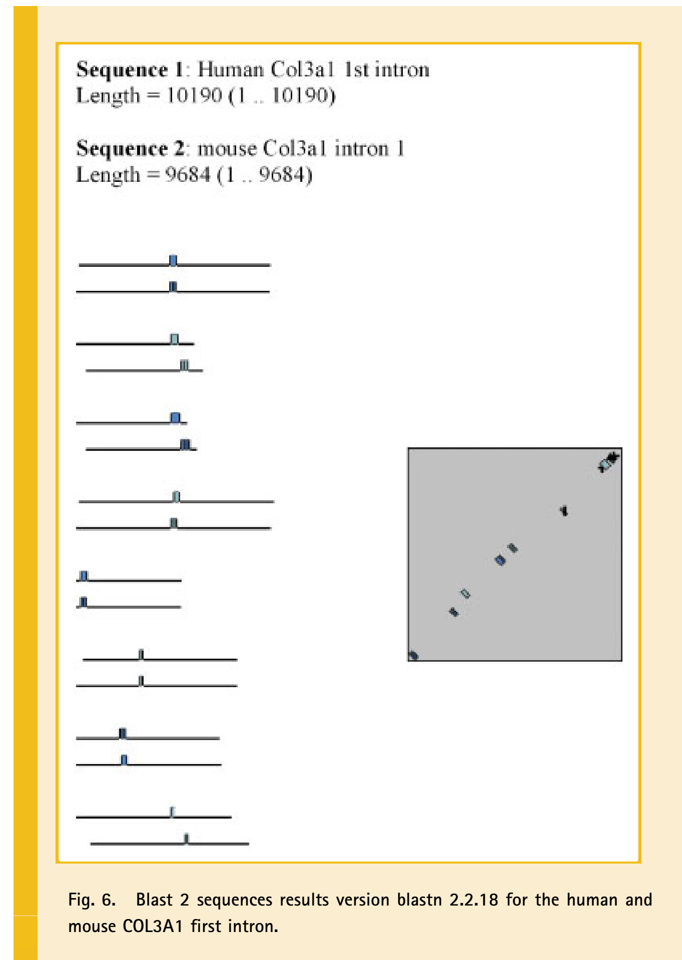

Fig. 6. Blast 2 sequences results version blastn 2.2.18 for the human and mouse COL3A1 first intron.

and a predicted reverse strand terminal exon and polyadenylation site (Fig. 4A). The rat, human, and chicken COL3A1 first introns also contain predicted promoters and exons (Fig. 4B–D).

Alignment of the mouse–rat and mouse–human COL3A1 first introns indicated approximately 84% sequence identity between the former (Fig. 5) and significantly less sequence identity between the latter (Fig. 6). There was no significant identity between the mouse and the chicken COL3A1 first introns.

A search of the miRBase Sequence Database with COL3A1 first intron sequences from each of the four species recognized several known mature miRNAs for the mouse, rat, human, and chicken [Glazov et al., 2008] (Fig. 4A–D). The miRBase Target database was searched for known or predicted gene targets of the COL3A1 first intron miRNAs. Predicted targets for mmu-miR-466f-3P include COL1A1, COL19A1, COL11A2, COL9A1, COL4A1, and COL4A5 (Fig. 7).

DISCUSSION

New miRNAs and their host genes are being reported on a regular basis [Li et al., 2007; Laurent et al., 2008]. The present description of COL3A1 transcripts, ESTs and miRNAs builds upon an observation made in 1986 focusing on collagen gene expression in developing rat intestine [Walsh et al., 1987] and presents experimental data and bioinformatics data indicating that the mouse COL3A1 first intron generates RNA transcripts that predominate in the suckling mouse intestine indicating developmental control of these transcripts. Support for this hypothesis comes from the fact that the mouse ESTs that overlap the 5.4 *Xba* I fragment of the mouse COL3A1 first intron are derived from fetal or developing tissues. Fetal and suckling mouse total and poly-A RNA were probed with an anti-sense RNA, hence only sense RNA transcripts were detected. However, the mouse, rat and human COL3A1 introns contain ESTs originating from the minus strand. The 3.0 kb transcript from fetal mouse is polyadenylated and corresponds to the mouse COL3A1 first intron region that has 98–100% sequence identity to a cluster of overlapping ESTs. At the time we were sequencing the mouse COL3A1 first intron (ca. 1988, data not shown) we observed unusual repeats, for example, CACACACACA . . . that piqued our interest. Toman and de Crombrugghe published the full sequence of the mouse COL3A1 gene in 1994 and subsequently we were able to search nucleotide databases for ESTs and miRNAs using the complete first intron sequence as we have presented here. The first intron of the mouse, rat, human, and chicken COL3A1 also contain predicted promoters, exons, and polyadenylation sites located on both plus and minus strands suggesting transcription by independent promoters other than that of the host gene. Mouse and rat ESTs CJ160307 and CN668922 contain sequence identity to the miRNAs mmu-miR-466f-3P, mmu-miR-574-5P, and mmu-miR-1187 (Table I, Fig. 4A).

```
A
Mouse CJ160307 inverted repeat

Score 49: 19/21 (90%) matches, 0 gaps

325 gtgtgtgtgtgtgtgtgtgtg 345
     |||||||||||| ||| ||||
404 cacacacacacagacagacac 384

B
CJ160307: 325-345, mmu-miR-466f-3p : 1-21, score: 87, evalue: 8.5

CJ160307            345  CACACACACACACACACACAC  325
                         || ||||||||||| ||||||
mmu-miR-466f-3p       1  CAUACACACACACAUACACAC  21

C
Position 1353-1359 of COL1A1 3' UTR  5'  ...UGUACCUAUUUUGUAUGUGUAUA...
                                                |||      |||||||
mmu-miR-466f-3p                      3'      CACACAUACACAC-ACACAUAC

Position 488-494   of COL4A5 3' UTR  5'  ...UGUAAGUAAAAGAAAUGUGUAUA...
                                                         |||||||
mmu-miR-466f-3p                      3'       CACACAUACACACACACAUAC

Position 1853-1859 of COL19A1 3' UTR 5'  ...GAAAAGUUGGUGCUAUGUGUAUA...
                                                         |||||||
mmu-miR-466f-3p                      3'       CACACAUACACACACACAUAC

Position 1207-1213 of COL4A1  3' UTR 5'  ...UGUAAUUUCUUUAAAUGUGUAUU...
                                                         |||||||
mmu-miR-466f-3p                      3'       CACACAUACACACACACAUAC

Position 2919-2925 of FNDC3B  3' UTR 5'  ...CUAACUGUAUCUGUA-GUGUAUAU...
                                               |||||      ||||||
mmu-miR-466f-3p                      3'       CACACAUACACACACACAUAC

Position 295-301   of COL11A2 3' UTR 5'  ...GAAGAGAAAGGACACUGUGUAUU...
                                                         |||||||
mmu-miR-466f-3p                      3'       CACACAUACACACACACAUAC
```

Fig. 7. A: Mouse CJ160307 inverted repeat. B: Sequence identity of CJ160307 to mmu-miR-466f-3p. C: Examples of predicted targets of mmu-miR-466f-3p.

TABLE I. Mouse COL3A1 1st Intron ESTs

Length (bp)	Overlapping mouse ESTs	COL3A1 1st intron position	Inverted repeat	miRNA
420	BB664446	5516–5890 bp	NF	NF
352	BY335218	5516–5865 bp	NF	NF
714	BB648452	1440–2116 bp	1–37, 224–260	ptc-miR-478 h mmu-miR-574-5p
508	CN668902	1930–2437 bp	7–20, 218–231	mmu-miR-1187
404	CJ160307	2093–2444 bp	325–345, 384–404	mmu-miR-466f-3p
705	BB534949	3013–3718 bp	277–293, 358–374, 495–511, 588–604,	NF
590	BG088384	3622–4210 bp		NF
596	AA543153	3766–4182 bp	68–90, 98–120	NF
391	BY671278	4828–5217 bp		NF
1012	DV075019	5002–5191 bp	312–327, 521–536	gga-miR-1651
636	BB363623	4641–5218 bp		NF
619	AW557338	5219–4601 bp	253–270, 518–501	NF
745	BG075843	5193–4474 bp		NF
121	CD546893	6521–6500 bp	NF	NF
668	CN688655	9402–9382 bp	NF	NF

NF, not found.
The Genbank accession number is given for each of the mouse ESTs (overlapping ESTs are grouped into separate rows) that were identified as corresponding to the mouse COL3A1 first intron in column 1. The length of the EST in base pairs is presented in column 2. Column 3 gives the position of the EST with respect to the mouse COL3A1 full-length gene. Column 4 indicates the position of inverted repeats identified within the EST with respect to its reported length (in base pairs). Column 5 indicates a mature miRNA identified for an EST.

TABLE II. Human COL3A1 1st Intron ESTs

Overlapping human ESTs	Length (bp)	COL3A1 1st intron position	Inverted repeat	miRNA
BG900720	712	5197–5939 bp	NF	NF
DB230813	599			
AL709928	683			
AL701285	391			
AL701594	594			
BX496725	388			
BX955745	608			
BX955750	589			
BX472623	642			
AW895750	469	6912–8481 bp	NF	NF
DA572628	849			
AU117551	771			
BX473342	660			
AF148885	253	9130–9404 bp	NF	NF
BI494551	231			
AW839433	217	6527–6447 bp	NF	NF
DR980741	101	5342–5250 bp	NF	NF
BQ008705	831	9224–8402 bp	308–341, 365–398	hsa-mir-1245
BQ025671	497			
AA989181	303			
AU144355	541	9404–8402 bp		
AU146808	544			
AW058627	453			
BQ007128	703			

NF, not found.
The length of the EST in base pairs is presented in column 2. Column 3 gives the position of the EST with respect to the human COL3A1 full-length gene. Column 4 indicates the position of inverted repeats identified within the EST with respect to its reported length (in base pairs). Column 5 indicates a mature miRNA identified for an EST. The Genbank accession number is given for each of the human ESTs (overlapping ESTs are grouped into separate rows) that were identified as corresponding to the human COL3A1 first intron in column 1.

The mmu-miR-466f-3P COL3A1 intronic miRNA has as its potential targets several other collagen genes (Fig. 7), one of which is type I collagen (COL1A1) a major collagen involved in fibrillogenesis with type III collagen [Liu et al., 1997]. The human COL3A1 first intron contains only one identified miRNA (hsa-mir-1245) for which no potential targets were found (Table II, Fig. 4C). Li et al. [2007] described the identification of multiple intronic miRNAs from the mouse and human genomes one of which (hsa-mir-455) was present in the COL27A1 intron 10. Their study also led them to hypothesize that intronic miRNAs may regulate the host gene's expression and/or that of proteins with which the host gene interacts. We originally hypothesized that the intronic transcripts described here are regulators of type III collagen expression however the present analysis did not indicate that type III collagen is a predicted COL3A1 intronic miRNA target. This does not exclude the possibility that the COL3A1 intronic transcripts regulate the host gene's expression since it has been reported that intronic sequences lacking an miRNA can regulate gene expression [Hill et al., 2006]. Also, bioinformatics analysis has identified several inverted repeats that may correspond to un-identified miRNAs. Several other collagen genes are targets for COL3A1 intronic miRNAs including type I collagen. Further study of the biological function of this and other COL3A1 miRNAs will be of significant importance since the possibility that type I collagen and other collagen genes are regulated by type III collagen first intron transcripts (miRNAs) provides an additional route for the regulation of collagen synthesis, fibrillogenesis, deposition and therapeutic intervention in disease, fibrosis and scarring. The role of collagen in development has been well documented [Hay, 1989] which is another avenue for investigation of regulatory pathways of collagen gene expression and its function in tissue differentiation. Future investigations with respect to COL3A1 first intron ESTs, inverted repeats and miRNAs and the regulation of other genes, specifically collagen genes will provide crucial information regarding tissue development and disease.

ACKNOWLEDGMENTS

This manuscript is dedicated to the memory of Professor Kenneth R. Cutroneo, a mentor and a friend. The author also wishes to thank Professor Rolf Renne for helpful discussions pertaining to miRNAs.

REFERENCES

Abend JR, Low JA, Imperiale MJ. 2010. Global effects of BKV infection on gene expression in human primary kidney epithelial cells. Virology 397: 73–79.

Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xlao H, Merril CR, Wu A, Olde B, Moreno RF, Kerlavage AR, McCombie RW, Venter C. 1991. Complementary DNA sequencing: Expressed sequence tags and human genome project. Science 252:1651–1656.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402.

Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschul T. 2003. A uniform system for microRNA annotation. RNA 9:277–279.

Bajic VB, Tan SL, Suzuki Y, Sugano S. 2004. Promoter prediction analysis on the whole human genome. Nat Biotechnol 11:1467–1473.

Bartel DP. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116:281–297.

Boss IW, Plaisance KB, Renne R. 2009. Role of virus-encoded microRNAs in herpesvirus biology. Trends Microbiol 17:544–553.

Burge C, Karlin S. 1997. Prediction of complete gene structures in human genomic DNA. J Mol Biol 268:78–94.

Byers PH. 2000. Collagens: Building blocks at the end of the development line. Clin Genet 58:270–279.

Chan T-F, Poon A, Basu A, Addleman NR, Chen J, Phong A, Byers PH, Teri E, Klein TE, Kwok P-Y. 2008. Natural variation in four human collagen genes across an ethnically diverse population. Genomics 91:307–314.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159.

Cummins JM, Velculescu VE. 2006. Implications of micro-RNA profiling for cancer diagnosis. Oncogene 25:6220–6227.

Gant TW, Petra R, Baus PR, Clothier B, Riley J, Davies R, Judah DJ, Edwards RE, George E, Greaves P, Smith AG. 2003. Gene expression profiles associated with inflammation, fibrosis, and cholestasis in mouse liver after griseofulvin. Environ Health Perspect 111:847–853.

Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM. 2006. MicroRNA expression and function in cancer. Trends Mol Med 12:580–587.

Glazov EA, Cottee PA, Barris WC, Moore RJ, Dalrymple BP, Tizard ML. 2008. A microRNA catalog of the developing chicken embryo identified by a deep sequencing approach. Genome Res 18:957–964.

Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. 2006. miRBase: MicroRNA sequences, targets and gene nomenclature. Nucleic Acids Res 34:D140–D144.

Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. 2008. miRBase: Tools for microRNA genomics. Nucleic Acids Res 36:D154–D158.

Hay ED. 1989. Extracellular matrix, cell skeletons, and embryonic development. Am J Med Genet 34:14–29.

Hill AE, Hong JS, Wen H, Teng L, McPherson DT, McPherson SA, Levasseur DN, Sorscher EJ. 2006. Micro-RNA-like effects of complete intronic sequences. Front Biosci 11:1998–2006.

Hillier LW, Graves TA, Fulton RS, Lucinda A, Fulton LA, Pepin KH, Minx P, Wagner-McPherson C, Layman D, Wylie K, Sekhon M, Becker MC, Fewell GA, Delehaunty KD, Miner TL, Nahs WE, Colin Kremitzki C, Oddy L, Du H, Sun H, Bradshaw-Cordum H, Ali J, Carter J, Cordes M, Harris A, Isak A, van Brunt A, Nguyen C, Du F, Courtney L, Kalicki J, Ozersky P, Abbott S, Armstrong J, Belter EA, Caruso L, Cedroni M, Cotton M, Davidson T, Desai A, Elliott G, Erb T, Fronick C, Gaige T, Haakenson W, Haglund K, Holmes A, Harkins R, Kim K, Kruchowski SS, Strong CM, Grewal N, Goyea E, Hou S, Levy A, Martinka S, Mead K, McLellan MD, Meyer R, Randall-Maher J, Tomlinson C, Dauphin-Kohlberg S, Kozlowicz-Reilly A, Shah N, Swearengen-Shahid S, Snider J, Strong JT, Thompson J, Yoakum M, Leonard S, Pearman C, Trani L, Radionenko M, Waligorski JE, Wang C, Rock SM, Tin-Wollam A-M, Maupin R, Latreille P, Wendl MC, Yang S-P, Pohl C, Wallis JW, Spieth J, Tamberlyn A, Bieri TA, Berkowicz N, Nelson JO, Osborne J, Ding L, Meyer R, Sabo A, Shotland Y, Sinha P, Wohldmann PE, Cook LL, Hickenbotham MT, Eldred J, Williams D, Jones TA, She X, Ciccarelli FD, Izaurralde E, Taylor J, Schmutz J, Myers RM, Cox DR, Huang X, McPherson JD, Mardis ER, Clifton SW, Warren WC, Chinwalla AT, Eddy SR, Marra MA, Ovcharenko I, Furey TS, Miller W, Eichler EE, Bork P, Suyama M, Torrents D, Waterston RH, Wilson RK. 2005. Generation and annotation of the DNA sequences of human chromosomes 2 and 4. Nature 434:724–731.

International Chicken Genome Sequencing Consortium. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432:695–716.

Kielty CM, Grant ME. The collagen family: Structure, assembly, and organization in the extracellular matrix. In: Royce PM, Steinmann B (Eds.) Connective tissue and its heritable disorders: Molecular, genetic, and medical aspects. New York: Wiley-Liss; 2002. pp 159–221.

Kloosterman WP, Plasterk RHA. 2006. The diverse functions of MicroRNAs in animal development and disease. Dev Cell 11:441–450.

Kuivaniemi H, Tromp G, Prockop DJ. 1997. Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. Hum Mutat 9:300–315.

Laurent LC, Chen J, Ulitsky I, Mueller F-J, Lu C, Shamir R, Fan J-B, Loring JF. 2008. Comprehensive MicroRNA profiling reveals a unique human embryonic stem cell signature dominated by a single seed sequence. Stem Cells 26: 1506–1516.

Li S-C, Tang P, Lin W-C. 2007. Intronic MicroRNA: Discovery and biological implications. DNA Cell Biol 26:195–207.

Liau G, Mudryaj M, de Crombrugghe B. 1985a. Identification of the promoter and first exon of the mouse alpha 1 (III) collagen gene. J Biol Chem 260: 3773–3777.

Liau G, Yamada Y, de Crombrugghe B. 1985b. Coordinate regulation of the levels of Type III and Type I collagen mRNA in most but not all mouse fibroblasts. J Biol Chem 260:531–536.

Lin S-H, Miller JD, Ying S-Y. 2006. Intronic MicroRNA (miRNA). J Biomed Biotechnol 2006:1–13. Article ID 26818.

Liu X, Wu H, Byrne M, Krane S, Jaenisch R. 1997. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. Proc Natl Acad Sci USA 94:1852–1856.

Maniatais T, Fritsch EF, Sambrook J. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Oleggini R, Musante L, Menoni S, Botti G, Duca MD, Prudenziati M, Carrea A, Ravazzolo R, Ghiggeri GM. 2000. Characterization of a DNA binding site that mediates the stimulatory effect of cyclosporin-A on type III collagen expression in renal cells. Nephrol Dial Transplant 15:778–785.

Pedersen AG, Baldi P, Chauvin Y, Brunak S. 1999. The biology of eukaryotic promoter prediction—A review. Comput Chem 23:191–207.

Pillai RS, Bhattacharyya SN, Filipowicz W. 2007. Repression of protein synthesis by miRNAs: How many mechanisms? Trends Cell Biol 17:118–126.

Rat Genome Sequencing Project Consortium. 2004. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature 428: 493–521.

Rice P, Longden I, Bleasby A. 2000. EMBOSS: The European Biology Molecular Open Software Suite. Trends Genet 16:276–277.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Toman PD, de Crombrugghe B. 1994. The mouse type-III procollagen-encoding gene: Genomic cloning and complete DNA sequence. Gene 147: 161–168.

Turashvili1 G, Bouchal J, Baumforth K, Wei W, Dziechciarkova M, Jiri Ehrmann J, Klein J, Eduard Fridman E, Skardal J, Srovnal J, Hajduch M, Murray P, Kolar Z. 2007. Novel markers for differentiation of lobular and ductal invasive breast carcinomas by laser microdissection and microarray analysis. BMC Cancer 7:55.

Verrecchia F, Mauviel A. 2007. Transforming growth factor-β and fibrosis. World J Gastroenterol 13:3056–3062.

Verrecchia F, Rossert J, Mauviel A. 2001. Blocking Sp1 transcription factor broadly inhibits extracellular matrix gene expression in vitro and in vivo: Implications for the treatment of tissue fibrosis. J Invest Dermatol 116:755–763.

Walsh MJ, LeLeiko NS, Sterling KM, Jr. 1987. Regulation of Type I, III and IV procollagen mRNA synthesis in glucocorticoid-mediated intestinal development. J Biol Chem 262:10814–10818.

Wu F, Chakravarti S. 2007. Differential expression of inflammatory and fibrogenic genes and their regulation by NF-kappaB inhibition in a mouse model of chronic colitis. J Immunol 179:6988–7000.