Human Type III Collagen Gene Expression Is Coordinately Modulated with the Type I Collagen Genes during Fibroblast Growth

Marcelle Miskulin,[‡] Raymond Dalgleish,[§] Barbara Kluve-Beckerman,[∥] Stephen I. Rennard,[⊥] Paul Tolstoshev,[#] Mark Brantly, and Ronald G. Crystal*

Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

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ABSTRACT: Type III collagen is one of the major interstitial collagens and, as such, plays an important role in modulating the structure and function of most tissues. To compare the expression of the type III collagen gene to that of the type I collagen $\alpha 1$(I) and $\alpha 2$(I) genes, cDNAs encoding the 3′ one-third of the human $\alpha 1$(III) collagen mRNA were obtained by screening a human fetal lung fibroblast cDNA library with a cloned segment of the chicken $\alpha 1$(III) gene. Northern blot analysis of human fetal lung fibroblast RNA demonstrated two $\alpha 1$(III)-specific mRNAs of sizes 6.6 and 5.8 kilobases, sizes clearly different from those of the type I collagen mRNAs. Analyses of populations of dividing and nondividing human lung fibroblasts revealed that, on a per cell basis, the nondividing population contained twice as much $\alpha 1$(III) mRNA than did the dividing population. The same was true for the type I collagen $\alpha 1$(I) and $\alpha 2$(I) mRNA transcripts. Similar results were obtained when $\alpha 1$(III), $\alpha 1$(I), and $\alpha 2$(I) mRNA transcripts were quantified by using dot blot evaluation of total RNA, Northern analysis of total RNA, and dot blot evaluation of cytoplasmic RNA. Thus, despite the fact that the $\alpha 1$(III) collagen gene is located on a chromosome different from the $\alpha 1$(I) and $\alpha 2$(I) genes, the expression of these three collagen chains appears to be coordinately controlled during periods of rapid and slow fibroblast growth.

Type III collagen is an interstitial collagen comprising 5–20% of the nonbasement membrane collagens of mammals (Bornstein & Sage, 1980; Miller & Gay, 1982). Although its function is not entirely understood, type III collagen is generally found in close association with the more abundant type I collagen and is thought to play an important role in defining tissue architecture and mechanical properties. The critical importance of this macromolecule in normal organ structure and function is dramatically illustrated in the Ehlers-Danlos type IV syndrome (Pope et al., 1973, 1983; Byers et al., 1981), an inherited disorder associated with a deficiency of type III collagen and characterized by fragile skin, aortic aneurysms, and intestinal rupture.

As an approach to understanding the regulation of type III collagen expression in humans, we have utilized a human fibroblast cDNA library to isolate recombinant plasmids that contain DNA complementary to the 3′-terminal one-third of human type III collagen mRNA. Using one of these plasmids as a probe, we have found the following: (1) on a per cell basis, a population of nondividing human fibroblasts contains twice as much $\alpha 1$(III) mRNA as when the same population is proliferating; and (2) despite the fact that the $\alpha 1$(III) gene is located on a different chromosome from both of the type I collagen genes, the modulation of $\alpha 1$(I), $\alpha 2$(I), and $\alpha 1$(III) mRNA levels is coordinately controlled during proliferating and nonproliferating conditions.

Materials and Methods

Materials. Elutip-d chromatography columns and nitrocellulose (BA85) were from Schleicher & Schuell. NACS PREPAC chromatography columns, a nick translation reagent kit, and *Escherichia coli* DNA polymerase Klenow fragment were obtained from Bethesda Research Laboratories. RNase T_1 was from Boehringer-Mannhein, and DNA sequencing reagents were from New England Biolabs. All other reagents were as previously described (Tolstoshev et al., 1981a,b; Dalgleish et al., 1982).

DNA Probes. Three cDNAs were used to evaluate chain-specific collagen mRNA expression. $\alpha 1$(I) mRNA was detected by using the larger *Eco*RI insert fragment of Hf677, a cloned human $\alpha 1$(I) cDNA (Chu et al., 1982). $\alpha 2$(I) mRNA was detected by using the 4.0-kilobase (kb) *Eco*RI fragment of HpC1, an exon-containing region of an $\alpha 2$(I) genomic clone (Dalgleish et al., 1982). $\alpha 1$(III) mRNA was detected by using $\alpha 1$(III) cDNAs isolated from a cDNA library prepared from diploid human lung fibroblasts (see below). The exon-containing region of the human $\alpha 1$(II) [previously referred to as "$\alpha 1$(I)-like"] genomic subclone pPstI (Weiss et al., 1982; Cheah et al., 1985) was used as a control for the isolation of the $\alpha 1$(III) cDNAs.

Isolation of $\alpha 1$(III) cDNAs. $\alpha 1$(III) cDNAs were prepared from RNA extracted from a human fetal lung fibroblast strain (HFL-1, American Type Culture Collection CCL 153) known to produce type I and type III collagens in a ratio of approximately 4:1. The cells were grown as previously described (Breul et al., 1980) and maintained at confluency for 2–3 days before removal from the cultures in a solution of 4 M guanidine thiocyanate. The RNA was isolated by a modification of the method of Chirgwin et al. (1979) in a procedure that included three extractions in 8 M guanidine hydrochloride, each followed by overnight ethanol precipitation of the RNA. The

* Address correspondence to this author.
[‡] Present address: Unité de Recherche de Metabolisme du Coeur et des Vaisseaux, Hopital Lariboisiere, Paris 10[e], France.
[§] Present address: Department of Genetics, University of Leicester, Leicester, LE1 7RH England.
[∥] Present address: Department of Medicine, Rheumatology Division, Indiana University Medical School, Indianapolis, IN 46223.
[⊥] Present address: Pulmonary Section, The University of Nebraska, Medical Center, Omaha, NE 68105.
[#] Present address: Biotechnology Australia Pty, Ltd., Roseville, NSW 2069 Australia.

RNA was dissolved in 20 mM ethylenediaminetetraacetic acid (EDTA), extracted with chloroformbutanol (4:1 v/v), precipitated with ethanol in 3 M sodium acetate, pH 5.0, and subsequently processed as previously reported (Tolstoshev et al., 1981b).

Poly(A+)-containing RNA was selected from this RNA preparation by two cycles of chromatography on poly(U)–Sepharose (Pharmacia) according to manufacturer's directions. With this template, double-stranded cDNA was prepared as described by Jaye et al. (1983). Molecules greater than 1000 base pairs (bp) were selected for cloning by sucrose gradient centrifugation, and the double-stranded cDNA was inserted into the *Pst*I site of pBR322 by G-C tailing. Screening of the cDNA library was performed by using methods described by Hanahan and Meselson (1983). The probe used for screening the cDNA library was derived from a subcloned segment of the chicken α1(III) genomic clone encoding exons 2 and 3 (Yamada et al., 1983a,b). The chicken exon 2 and exon 3 probe was purified free of vector DNA by restriction enzyme digestion and two cycles of purification by preparative electrophoresis in agarose. Agarose and other contaminants were removed by chromatography on an Elutip-d chromatography column. The probe DNA was labeled with [^{32}P]dCTP by nick translation (Rigby et al., 1977). Filters were prewashed at 65 °C for 4 h in 3× SSC (20× SSC = 3.0 M sodium chloride and 0.3 M sodium citrate, pH 7.0), 5× Denhardt's solution, 200 μg/mL herring sperm DNA (sonicated and denatured), 50 μg/mL *E. coli* DNA (sonicated and denatured), and 0.1% sodium dodecyl sulfate (SDS). Hybridization was at 65 °C for 18 h in 3× SSC, 2× Denhardt's solution, 200 μg/mL herring sperm DNA, and 50 μg/mL *E. coli* DNA (each sonicated and denatured). After hybridization, the filters were washed for 3 h at a final stringency of 2× SSC and 0.1% SDS, 65 °C to allow for the expected mismatch between human and chicken α1(III) gene sequences. Autoradiography was accomplished by using intensifying screens in liquid nitrogen vapor. Approximately 60 positive colonies were obtained consisting of roughly equal numbers of "strong" and "weak" positives. Two strong positive colonies designated pIII-21 and pIII-33, each containing inserts of 1.9 kb in length, were selected for further investigation.

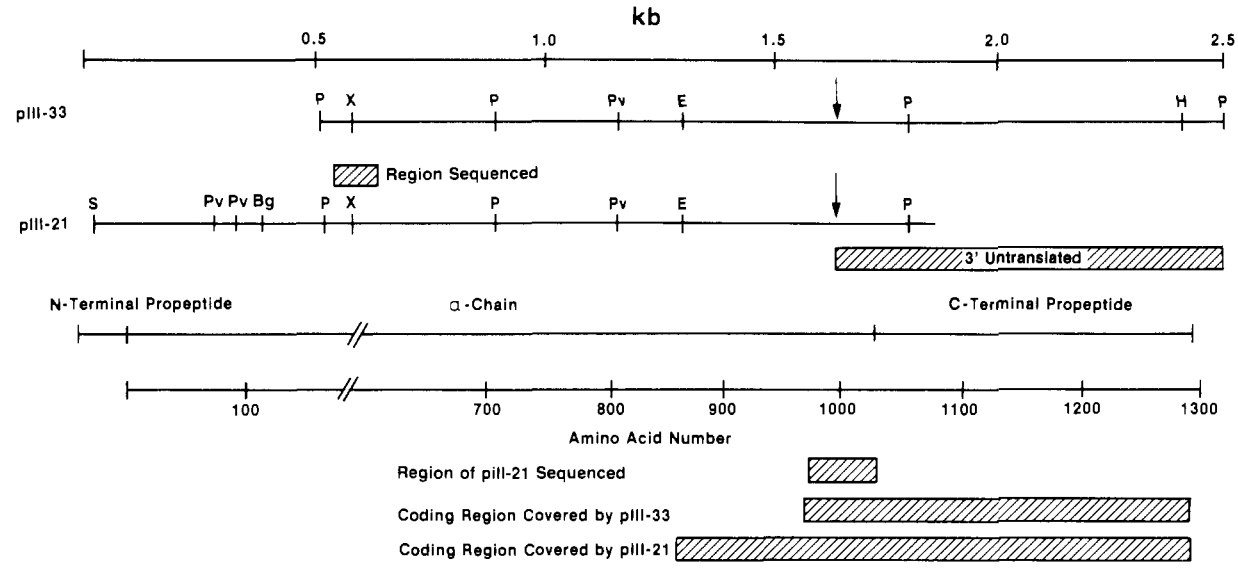
Plasmid DNA was prepared for mapping purposes and subcloning by lysozyme treatment and detergent lysis followed by CsCl density gradient centrifugation essentially as described by Clewell and Helinski (1969). For restriction endonuclease mapping, DNA was blotted from agarose gels as described by Southern (1975). Washing and hybridization were as described for colony hybridization but without the *E. coli* DNA. Restriction mapping of the two cDNA clones, pIII-33 and pIII-21, demonstrated they were overlapping (Figure 1). Identification of these clones as α1(III) cDNA was made by DNA sequencing of one of the clones, pIII-21, using the dideoxy chain termination method with adaptions for the use of double-stranded DNA as template (Sanger et al., 1977; Smith et al., 1979; Wallace et al., 1981). The DNA to be sequenced was subcloned into the *Pst*I site of the plasmid vector pUC9 (Vieira & Messing, 1982). Recombinant plasmid DNA was prepared from 10 mL of a saturated culture of bacteria by a rapid alkaline lysis procedure (Ish-Horowitz & Burke, 1981). RNA was removed by digestion with RNase T_1 followed by chromatography on a NACS PREPAC column according to manufacturer's directions. The DNA was made linear by digestion with the restriction enzyme *Sma*I. The advantages of using this enzyme were that, with respect to the primer, *Sma*I cuts the vector in the poly-linker region distal to the *Pst*I site which contains the insert and that there are no *Sma*I sites in the 340 bp fragment of pIII-21. The DNA was then incubated with M13 reverse sequencing primer at a 4-fold higher ratio of primer to template than is used in the sequencing of single-stranded DNA. Primer and template were sealed into a glass capillary tube, boiled for 3 min, and then quenched in ice/water. Other steps were carried out by standard procedures. DNA sequence analysis was carried out by using the SEQ program (Brutlag et al., 1982) on the DEC-10 computer at the Division of Computer Research and Technology, NIH.

To orient the α1(III) cDNAs, fragments of pIII-33 were isolated, labeled by nick translation, and individually hybridized to 5′ and 3′ regions of the chicken α1(III) gene. The regions of the chicken gene used were *Eco*RI digest fragments of the clone λC3-C1-24 (Yamada et al., 1983a,b) subcloned in pBR322; these regions encoded exon 4, exons 3 and 2, and exon 1 of the chicken α1(III) gene, respectively. The subcloned fragments were digested with *Eco*RI, electrophoresed in agarose, blotted bidirectionally (Smith & Summers, 1980) onto duplicate nitrocellulose filters, and used as targets for the ^{32}P-labeled *Pst*I fragments of pIII-33.

Cell Cultures. Two cell types were evaluated, RD rhabdomyosarcoma (RMS) cells (American Type Culture Collection CCL 136) and HFL-1 diploid human fetal lung fibroblasts (American Type Culture Collection CCL 153). The RMS cells are a transformed line that produces α1(III) collagen chains and only very small amounts of type I collagen (Krieg et al., 1979) and thus could be used to demonstrate the specificity of the α1(III) probe. The HFL-1 cells produce α1(I), α2(I), and α1(III) collagen chains in an approximate ratio of 6:4:1 and produce equal amounts of collagen during periods of slow and rapid growth, respectively (Breul et al., 1980; Hance & Crystal, 1977; Tolstoshev et al., 1981a,b; Rennard et al., 1982). The cells were cultured as previously described (Breul et al., 1980). The RD cells were evaluated at confluency and the HFL-1 cells at log phase (4×10^2 cells/mm^2) and confluency. In parallel cultures, the rate of type I and type III collagen production was quantified as previously described (Breul et al., 1980; Hance & Crystal, 1977; Tolstoshev et al., 1981a,b; Rennard et al., 1982), and collagen α1(I), α2(I), and α1(III) mRNA levels were quantified as described below.

Quantification of Collagen Gene Expression. Three methods were utilized to quantify collagen gene expression at the mRNA level: (1) Northern blot hybridization to total cellular RNA; (2) dot blot hybridization to total cellular RNA; and (3) dot blot hybridization to cytoplasmic RNA. For all three methods, the data were expressed on a per cell basis using the ratio of RNA to DNA for each culture condition as indicated. In all cases, the probes were labeled with ^{32}P by nick translation.

For the Northern blot analysis, total RNA was harvested from the cell cultures according to the guanidine thiocyanate method (Chirgwin et al., 1979). The RNA was dissolved in 10 mM sodium phosphate, pH 7.4, 50% formamide, 2.2 M formaldehyde, and 0.5 mM EDTA and denatured by heating at 65 °C for 5 min. A one-fifth volume of loading buffer (0.5% SDS, 0.025% bromophenol blue, 25% glycerol, and 25 mM EDTA) was added and the RNA fractionated by electrophoresis in 1% agarose for 5 h at 60 V. The gel and electrophoresis buffer contained 1.1 M formaldehyde and 10 mM sodium phosphate, pH 7.4. Transfer of RNA to nitrocellulose was in 20× SSC according to the method of Thomas (1980). Hybridization and washing were for 40 h, and the final wash



	990									1000									1010
Human α1(III) cDNA Sequence	GGA	CCA	CCA	GGG	CCT	CGA	GGT	AAC	AGA	GGT	GAA	AGA	GGA	TCT	GAG	GGC	TCC	CCA	GGC	CAC	CCA	GGG	CAA	CCA	GGC
Translated Sequence	Gly	Pro	Pro	Gly	Pro	Arg	Gly	Asn	Arg	Gly	Glu	Arg	Gly	Ser	Glu	Gly	Ser	Pro	Gly	His	Pro	Gly	Gln	Pro	Gly
Human α1(III) Amino Acid Sequence	Gly	Pro	Hyp	Gly	Pro	Arg	Gly	Asn	Arg	Gly	Glu	Arg	Gly	Ser	Glu	Gly	Ser	Hyp	Gly	His	Hyp	Gly	Gln	Hyp	Gly

	1020								1023					
	CCT	CCT	GGA	CCT	CCT	GGT	GCC	CCT	GGT	CCT	TGC	TGT	GGT	GGT	GTT
	Pro	Pro	Gly	Pro	Pro	Gly	Ala	Pro	Gly	Pro	Cys	Cys	Gly	Gly	Val
	Pro	Hyp	Gly	Pro	Hyp	Gly	Ala	Hyp	Gly	Pro	Cys	Cys	Gly	Gly	Val

FIGURE 1: Map of the human α1(III) cDNA clones isolated from a human diploid lung fibroblast cDNA library. The scale at the top indicates size in kilobases. Two clones, pIII-33 and pIII-21, were evaluated. Sites for the restriction enzymes *Sst*I, *Pst*I, *Eco*RI, *Xho*I, *Pvu*II, *Bgl*I, and *Hin*dIII are indicated as S, P, R, X, Pv, Bg, and H, respectively. The shaded block above pIII-21 indicates the region sequenced. The arrows indicate the likely stop codon site; the shaded area 3′ to this site represents the untranslated sequence of pIII-21. Shown below the cDNAs is a representation of the pro-α1(III) collagen chain indicating the regions represented by clones pIII-33 and pIII-21 as well as the region of pIII-33 sequenced. The sequences shown are those of pIII-21, the in-phase translated sequence of pIII-33, and the sequence of the human α1(III) chain (Seyer & Kang, 1981). Hyp indicates proline residues that have been hydroxylated posttranslationally. The numbering system used for the amino acids is that of Seyer and Kang (1981).

was in 0.2× SSC and 0.1% SDS. The filters were hybridized with probes for human α1(I), human α2(I), and human α1-(III) collagen. The labeled RNA bands were then evaluated by autoradiography. For the quantitative evaluation for the HFL-1 cells, the autoradiograms were scanned with a densitometer. Autoradiograms with different doses of RNA showed that the autoradiograms were in the linear response range of the X-ray film. The results are expressed as arbitrary densitometer units per cell.

For the dot blot hybridization to total cellular RNA, the RNA was extracted as described above for the Northern analysis. The extracts were diluted at various concentrations (0.28–28 μg) of total RNA with a solution of 6.15 M formaldehyde/10× SSC. RNA was denatured at 65 °C for 15 min and applied to nitrocellulose sheets (Schleicher & Schuell BA85; 0.45 μm) by employing a minifold apparatus (Schleicher & Schuell). Then nitrocellulose was baked 80 °C for 90 min to fix the macromolecules. The efficiency of RNA binding to the nitrocellulose was determined by adding [^{3}H]RNA to the RNA preparations in parallel dots and measuring the amount of radioactivity after the RNA was fixed. Under the conditions used, 92 ± 4% of the radioactivity was recoverd. Hybridization to the dots was carried out as for the Northern analysis as was the quantification of the signals.

For the dot blot hybridization to cytoplasmic RNA, the cytoplasmic RNA was recovered by the method of White and Bancroft (1982). Briefly, the cells were washed with DMEM supplemented with 50 units/mL placental ribonuclease inhibitor. The cells were then harvested with 2 mL of 0.25% trypsin (5 min, 25 °C), centrifuged with 20% fetal calf serum and 50 units/mL placental ribonuclease inhibitor, washed 3 times with phosphate-buffered saline, pH 7.4, and 50 units/mL placental ribonuclease inhibitor, and pelleted (1200*g*, 10 min, 4 °C). The cell pellet was homogenized twice in a cold lysis buffer [10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.0, 1 mM EDTA, 0.5% Nonidet P40, and 50 units/mL placental ribonuclease inhibitor]. The nuclei were pelleted by centrifugation (5000*g*, 2.5 min, 4 °C); phase contrast microscopic evaluation revealed well-preserved nuclei. The supernatant was used as the preparation of cytoplasmic RNA. As a control, two aliquots were used, one with and one without RNase A (20 units/mL). Both aliquots were incubated for 1 h, 37 °C, mixed with 6× SSC, 7.4% (w/v) formaldehyde, and 50 units/mL placental ribonuclease inhibitor, heated at 65 °C for 15 min, and then stored at −70 °C until evaluation. Hybridization and quantitation of the cytoplasmic RNA dots were carried out as described above.

Biohazard Precautions. All manipulations of recombinant DNA were carried out in accordance with NIH guidelines for recombinant DNA research.

RESULTS

Characterization of α1(III) cDNAs. Two plasmids containing α1(III) cDNAs were evaluated: pIII-33 and pIII-21, each containing a 1.9-kb insert. Restriction enzyme analysis demonstrated the inserts represented overlapping sequences (Figure 1). The following enzymes did not cut the inserts:

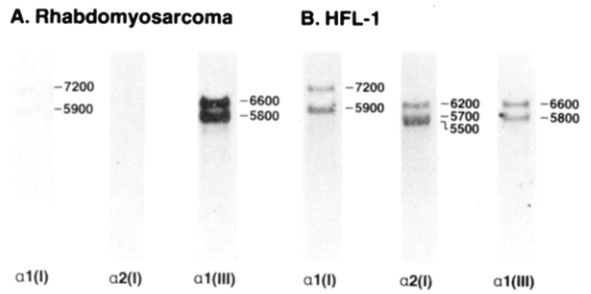


FIGURE 2: Northern blot analysis of RNA isolated from rhabdomyosarcoma and HFL-1 fibroblast cell lines and evaluated with human $\alpha 1(I)$, $\alpha 2(I)$, and $\alpha 1(III)$ collagen gene probes. (A) Total RNA (4 μg) from a rhabdomyosarcoma cell line was electrophoresed in 1% agarose containing 1.1 M formaldehyde onto nitrocellulose and hybridized with ^{32}P-labeled cloned DNA probes specific for the human $\alpha 1(I)$, $\alpha 2(I)$, and $\alpha 1(III)$ genes as indicated. (B) Total RNA from HFL-1 cells treated as described in (A). Sizes (in bases) for the $\alpha 1(I)$ and $\alpha 2(I)$ mRNAs detected in (A) and (B) are taken from Myers et al. (1981) and Chu et al. (1982). The sizes of the $\alpha 1(III)$ transcripts were determined by comparison with those for $\alpha 1(I)$ and $\alpha 2(I)$.

*Bcl*I, *Bgl*II, *Hpa*I, *Kpn*I, *Sma*I, *Sst*II, and *Xba*I. Hybridization to a cloned $\alpha 1(I)$ cDNA, $\alpha 2(I)$ genomic DNA, and to $\alpha 1(II)$ DNA indicated that pIII-33 and pIII-21 did not contain type I or type II collagen gene sequences (data not shown). To determine the orientation of the cloned DNAs with respect to the protein sequence for which they code, three fragments (380, 925, and 670 bp, 5′ to 3′ *Pst*I fragments in Figure 1) of the insert of pIII-33 were isolated to determine which fragment hybridized to the different regions of the chicken type III collagen gene. The 380 bp fragment hybridized to the 5′ region of the chicken gene, and the 670 bp fragment was 3′ to the 925 bp fragment, thus establishing the orientation of the clone as indicated (data not presented).

To formally establish the identity of the cDNAs as complementary to the human $\alpha 1(III)$ collagen gene, a portion of pIII-21 was sequenced and compared to the known sequence of the human $\alpha 1(III)$ chain (Figure 1). When the sequence was translated in all three reading frames, it was apparent that translation in the first reading frame revealed the repeating Gly-X-Y motif typical of collagen. Furthermore, the derived amino acid sequence corresponded exactly with that established for human type III collagen by amino acid sequencing (Seyer & Kang, 1981), including the Cys-Cys pair (amino acids 1022–1023) that is unique among the collagens, for $\alpha 1(III)$ chains. In addition, comparison of the sequence of pIII-21 to the sequence of the type III procollagen cDNA clones RJ5 and E6 characterized by Loidl et al. (1984), and the type III procollagen exon sequence determined by Chu et al. (1985), demonstrated (1) an exact correspondence with RJ5 and E6 and (2) a difference of 2 bp when compared with the results of Chu et al. (1985).

When the nucleotide sequence of pIII-21 was compared with that of the corresponding region (exons 5 and 4) of the chicken $\alpha 1(III)$ collagen gene, a great deal of homology was apparent at both the nucleotide and amino acid levels. There was 74% homology between the sequences at the nucleotide level with 55% of these differences silent. At the amino acid level, there was an 81% correspondence. Three triplets, coding for Gly-Ser-Pro (corresponding to residues 1003–1005 of the human helical region) present in the human genome, were not present in the chicken $\alpha 1(III)$ gene.

$\alpha 1(III)$ Collagen Gene Expression Compared to $\alpha 1(I)$ and $\alpha 2(I)$ Gene Expression. Northern analysis of $\alpha 1(III)$ mRNA in two very different fibroblasts, HFL-1, a diploid fibroblast producing three major collagen chains [$\alpha 1(I)$, $\alpha 2(I)$, and $\alpha 1(III)$], and RMS, a transformed fibroblast producing almost

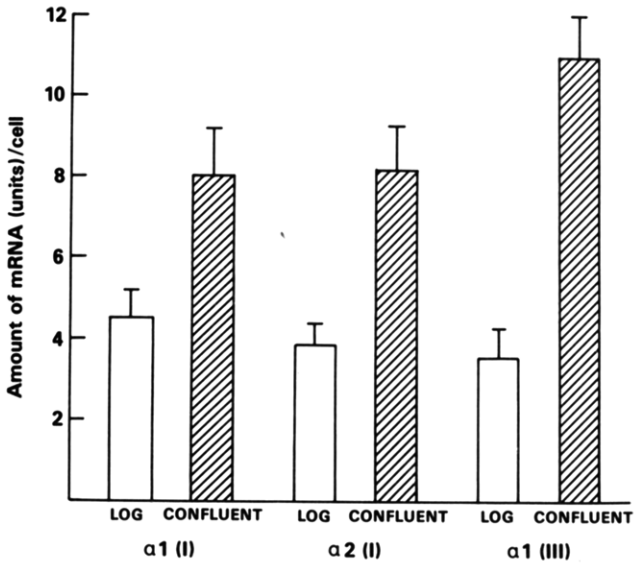


FIGURE 3: Quantification of the relative amounts of $\alpha 1(III)$ mRNA present in HFL-1 fibroblasts during different growth conditions. Total RNA (2 μg) isolated from rapidly dividing ("log") and nondividing ("confluent") HFL-1 cells was evaluated with the dot blot method by applying the RNA directly to nitrocellulose and hybridizing with ^{32}P-labeled $\alpha 1(I)$, $\alpha 2(I)$, and $\alpha 1(III)$ specific DNA probes. Autoradiographic images of the hybridizations were quantified by densitometry and expressed in arbitrary units per cell. All studies were done in triplicate. Standard errors of means are indicated.

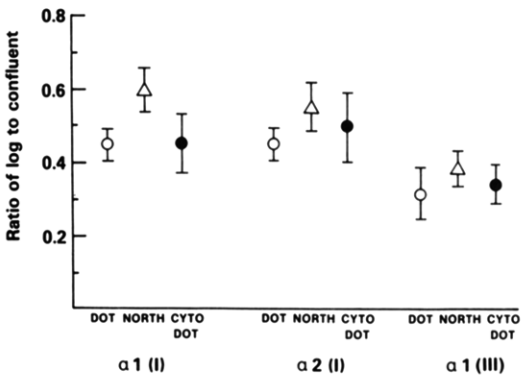


FIGURE 4: Ratio of the amounts of collagen chain mRNAs in fibroblasts in log phase of growth to that in confluency. Data from these methods of measurement are compared: "DOT" = analysis of total RNA by dot hybridiztion (these data are derived from those in Figure 3); "NORTH" = analysis of total RNA by Northern hybridization; and "CYTO DOT" = analysis of cytoplasmic RNA by dot hybridization. All studies were done in triplicate. Standard errors of means are indicated.

entirely $\alpha 1(III)$ chains, demonstrated that (1) $\alpha 1(III)$ mRNA was present in both cell types as two species, one of 6.6 kb and one of 5.8 kb, and (2) the two $\alpha 1(III)$ mRNAs were of different sizes than the $\alpha 1(I)$ and $\alpha 2(I)$ mRNAs (Figure 2). As expected, the types of mRNAs present dictated the spectrum of collagens produced by these cells. The RD cells contained large amounts of $\alpha 1(III)$ mRNA, no detectable $\alpha 2(I)$ mRNA, and barely detectable $\alpha 1(I)$ mRNA. In contrast, the HFL-1 cells contained easily detectable $\alpha 1(I)$, $\alpha 2(I)$, and $\alpha 1(III)$ mRNAs. Interestingly, the amount of $\alpha 1(III)$ mRNA per cell was greater in the RD cells than in the HFL-1 cells (data not shown).

At the protein level, HFL-1 cells produce equal amounts of $\alpha 1(III)$ molecules per cell during periods of rapid growth [9.5 ● 1.5 molecules $(10^6 \text{ cells})^{-1}\ h^{-1}$] and confluency [10.5 ● 1.2 molecules $(10^6 \text{ cells})^{-1}\ h^{-1}$; $p > 0.1$). On the average, however, the confluent cells contained almost twice the number of $\alpha 1(III)$ mRNA copies per cell than did the log-phase cells

(Figures 3 and 4; $p < 0.01$). Thus, the expression of the α1(III) gene at the protein level presumably involves mechanisms in addition to the number of α1(III) mRNA copies per cell. Interestingly, the two mRNA transcripts of the α1(III) gene were evident in both the confluent and log-phase cells. However, although the ratio among the two transcripts appeared to fall from approximately 2 to 1 (ratio of amount of small transcripts to large transcripts) to 1 to 1 as the cells moved from log phase to confluency, the variability of the methodology used to evaluate these separate transcripts makes it impossible, at this time, to determine if this is a significant change.

Strikingly, the enhanced number of α1(III) gene transcripts found in the confluent cells was paralleled by increased amounts of α1(I) and α1(2) gene transcripts (Figures 3 and 4). For all three collagen genes, the log-phase cells expressed 40–60% of the α1(I), α2(I), and α1(III) mRNA transcripts as did the confluent cells. Furthermore, this observation was true independent of the method used to obtain and analyze the RNA (Figure 4, $p > 0.1$, all comparisons). Thus, not only does the number of collagen mRNA transcripts vary during periods of rapid and slow growth but also the modulation of all three collagen genes appears to be coordinately controlled.

Discussion

Type III collagen, comprised of three α1(III) chains, is the second most abundant interstitial collagen of most tissues and, as such, plays a major role in modulating tissue structure and function in health and disease (Muller et al., 1977; Pope et al., 1973, 1983; Byers et al., 1981). By use of a cDNA complementary to the C-terminal portion of the α1(III) collagen chain, the present study demonstrates that the number of type III collagen mRNA transcripts present in diploid human fibroblasts doubles when the cells go from a state of active proliferation to one of quiescent, slow growth. Importantly, this modulation of α1(III) mRNA transcript number occurs in parallel with that of the mRNA transcripts of both the α1(I) and α1(2) chains of type I collagen. Thus, despite the fact that the genes for these three collagen chains are found on different chromosomes (Solomon et al., 1985; Emanuel et al., 1985), the expression of α1(III), α1(I), and α1(2) collagen genes appears to be coordinately controlled, at least during periods of rapid and slow cell growth.

α1(III) Collagen Gene Expression. Evaluation of two disparate cells, the RD rhabdomyosarcoma and the HFL-1 diploid fetal lung fibroblast, demonstrated that the α1(III) collagen gene transcripts are two species of mRNA of equal amount. This observation is consistent with that noted by Loidl et al. (1984) and Chu et al. (1985), who also found two mRNA transcripts of the α1(III) collagen gene. Interestingly, Chu et al. (1985) have pointed out that the sequences of the α1(III) cDNA and genomic α1(III) clones suggest that the two α1(III) mRNA transcripts are colinear, with their size difference resulting from alternative transcription, termination, or processing points in the 3′ untranslated region. As we and others (Adams et al., 1979; Myers et al., 1983; Loidl et al., 1984; Chu et al., 1985) have noted, size polymorphism is a common feature of human procollagen mRNA.

Interestingly, although the production of collagen types I and III is constitutive during periods of rapid and slow growth, the number of intact mRNA transcripts for these type III collagen α1(III) chains as well as the type I collagen α1(I) and α2(I) chains changes markedly depending on the growth status of the cells. Strikingly, however, the changes for all three types of procollagen mRNAs occur in parallel, with each found in approximately 2-fold quantities during confluency. These observations, using filter hybridization methods, are consistent with our previous observations in the same cell strain using solution hybridization (Tolstoshev et al., 1981). Importantly, the fact that identical results were obtained with Northern analysis points out not only that the α1(III), α1(I), and α2(I) mRNA transcripts are in greater number during confluency but also that these are intact transcripts. Although the methodology does not permit sufficiently accurate quantification of the ratio of the two sizes of α1(III) mRNA to determine if there is variation in their relative abundance as the cell growth rate is altered, it is conceivable that the processing, stability, or efficiency of translation may vary for the two transcripts during log phase and confluency. Independent of these considerations, however, in view of the fact that the α1(III) gene is located on chromosome 2 (Solomon et al., 1985; Emanuel et al., 1985), the α1(I) gene on chromosome 17 (Burke et al., 1977; Heurre et al., 1982; Miller et al., 1982; Solomon et al., 1984; Tate et al., 1982), and the α2(I) gene on chromosome 7 (Junien et al., 1982; Myers et al., 1983; Solomon et al., 1983; Chu et al., 1984), the observation that the number of intact α1(III), α1(I), and α2(I) mRNA transcripts changes coordinately suggests that collagen-producing cells must have some mechanisms to modulate these widely separate genes together or to handle the processing of the gene transcripts in a parallel fashion.

Acknowledgments

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