Different Levels of Regulation Accomplish the Switch from Type II to Type I Collagen Gene Expression in 5-Bromo-2'-deoxyuridine-treated Chondrocytes

G. Roger Askew†, Sandia Wang, and Lewis N. Lukens§
From the Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06459

The shift of chick embryo chondrocytes to a fibroblastic phenotype by 5-bromo-2'-deoxyuridine (BrdUrd) has been used to examine the molecular basis of the switch from type II to type I collagen gene expression. Transcription rates of each of these three collagen genes before and after this shift were measured in nuclear run-on transcription assays with double-stranded 3'-cDNA probes specific for each of these three mRNAs. Degradation rates of each of these RNAs were calculated from the rate of decrease in the concentration of each RNA after the inhibition of synthesis with actinomycin D. The shut-off of the expression of the type II collagen gene during this shift was shown to occur at the transcriptional level, since the transcription rate of this gene decreased dramatically. The decay rate of the type II mRNA (half-life of approximately 15 h) is not significantly faster in BrdUrd-treated cells. The α1(I) gene is transcribed at similar rates in untreated and shifted chondrocytes, but the steady state level of α1(I) RNA in chondrocytes is only 1.5% of that in shifted cells. Although the measured degradation rate of the total α1(I) RNA from untreated chondrocytes is approximately the same as in shifted cells (half-life of approximately 12 h), indirect evidence suggests that this α1(I) RNA is derived from a low level of fibroblast contamination of these chondrocyte cultures. The α1(I) RNA synthesized by untreated chondrocytes is assumed to be therefore broken down very rapidly in the nucleus. The α2(I) gene is also transcribed in untreated chondrocytes at rates similar to shifted cells but, unlike α1(I) RNA, its steady state level in untreated chondrocytes is approximately 30% of its level in shifted chondrocytes. The increased level of α2(I) RNA in shifted cells may be regulated in part by an increase in stability of the α2(I) mRNA, which has half-lives of 5.2 and 10.4 h, respectively, in untreated and shifted chondrocytes. The α2(I) RNA in the untreated chondrocytes was found to have a different 5' end from that present in the BrdUrd-shifted chondrocytes or in chick embryo fibroblasts. The presence of this altered RNA in untreated chondrocytes explains the absence of synthesis of the fibroblastic α2(I) collagen polypeptide chains in these chondrocytes, despite the presence of the α2(I) RNA as measured with 3' probes.

The expression of the collagen type I and type II genes is tightly regulated during embryonic development, with each type of collagen being made in specific tissues at specific times (1, 2). Type I collagen is relatively widely distributed, being present in bone, skin, tendons, and ligaments, whereas type II collagen is located almost exclusively in hyaline cartilage. A useful system for investigating the molecular mechanisms that regulate the expression of these genes is provided by the 5-bromo-2'-deoxyuridine (BrdUrd)-induced shift from type II to type I collagen synthesis in primary cultures of chondrocytes (3, 4). Primary cultures of embryonic chick sternal chondrocytes synthesize the specialized products characteristic of differentiated chondrocytes, including type II collagen. However, exposure of these cells to low levels of BrdUrd for 8 to 10 days causes these cells to lose their chondrocyte phenotype, so that they no longer synthesize type II collagen or other cartilage-specific products. The previously floating chondrocytes attach to the bottom of the culture dish, and they now synthesize a number of proteins, notably type I collagen and fibronectin, that are characteristic of fibroblasts. Since the mesenchymal cells that give rise to chondrocytes synthesize type I collagen (5), it is possible that this shift to a fibroblastic phenotype represents a dedifferentiation back to the mesenchymal phenotype. A similar shift in phenotype can be induced by the tumor promoter, phorbol-12-myristate-13-acetate (6, 7), or by repeated subculture of the chondrocytes in monolayer (reviewed in Ref. 5) or by their transformation with Rous sarcoma virus (8–10).

Our previous studies of the changes in steady state levels of the type I and type II collagen mRNAs in nucleus and cytoplasm during the BrdUrd shift suggested that the primary control of the type II gene was at the transcriptional level (11). The level of type II RNA fell sharply in BrdUrd-treated cultures, to undetectable levels in the nucleus by day 6 and in the cytoplasm by day 8. The type I mRNAs, however, appeared to be present at appreciable levels in the nucleus of untreated chondrocytes, an observation that suggested that the type I genes were transcribed in untreated chondrocytes, although these cells do not synthesize type I collagen. An additional unexpected finding was that the level of α2(I) mRNA in the cytoplasm of untreated chondrocytes, as measured with cDNAs from the 3' end of the mRNA, was relatively high, being one-third of its level in shifted cells, although

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‡ Present address: Dept. of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, 231 Bethesda Ave., Cincinnati, OH 45267.
§ To whom correspondence and reprint requests should be addressed.

1 The abbreviations used are: BrdUrd, 5-bromo-2'-deoxyuridine; kb, kilobase pair(s); bp, base pair(s); nt, nucleotides; PCR, polymerase chain reaction.
untreated chondrocytes do not synthesize \( \alpha_2(I) \) collagen chains. The level of cytoplasmic \( \alpha_1(1) \) mRNA was found, as expected, to be very low in chondrocytes relative to shifted cells (less than 3%).

To assess the relative contributions of changes in transcription and degradation rates to the control of the levels of these collagen mRNAs during the BrdUrd-induced shift, measurements of transcription rates and degradation rates for each mRNA were undertaken. The results reported here confirm the conclusion that the expression of the type II collagen gene is controlled primarily at the transcriptional level. Both type I collagen genes, however, appear to be transcribed in chondrocytes at rates comparable to the BrdUrd-shifted cells. The control of these genes is, therefore, not primarily due to changes in transcription rates. In the case of the \( \alpha_1(1) \) gene, indirect evidence suggests that the control is primarily at the level of nuclear stability. The \( \alpha_2(I) \) RNA shows relatively minor changes in the transcription rate but a significant increase in stability during the BrdUrd-induced shift. The chondrocyte form of the \( \alpha_2(I) \) RNA was found to have a different 5' than the 5' end present in fibroblasts or in the BrdUrd-shifted cells, in agreement with the discovery of Adams et al. (12, 13). Splicing of the chondrocyte-specific first exon, exon A, to exon 3 of the usual fibroblastic \( \alpha_2(I) \) mRNA disrupts the collagenous reading frame (13), so this altered \( \alpha_2(I) \) RNA does not code for collagen sequences. Therefore, expression of the \( \alpha_2(I) \) gene in chondrocytes and BrdUrd-shifted cells is regulated by choice of the transcription start site.

**MATERIALS AND METHODS**

Cell Culture—Chondrocytes were isolated from the sterna of 14-day-old chick embryos (a gift from Arbor Acres, Glastonbury, CT or purchased from Spafas, Norwich, CT) by the floater selection method of Schlitz et al. (14), except that Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum ( Gibco Laboratories) was used. The chondrocytes were induced to shift to a fibroblastic phenotype by culture in the presence of 6.5 × 10^{-3} M bromodeoxyuridine (Boehringer Mannheim).

Cloned cDNAs—Collagen cDNA subclones were constructed in the plasmid vector pBS (Stratagene) for use in nuclear run-on experiments and as templates for the synthesis of the antisense [\( ^{32}P \)]RNA probes used in the RNase protection assays. To avoid cross-hybridization caused by the sequence homology between these genes, the cloned cDNA probes were prepared from regions of minimal homology, and the homopolymer stretches in the 3' ends of these mRNAs were removed. The \( \alpha_2(I) \) cDNA probe is specific first exon, exon A, to exon 3 of the usual fibroblastic \( \alpha_2(I) \) mRNA disrupts the collagenous reading frame (13), so this altered \( \alpha_2(I) \) RNA does not code for collagen sequences. Therefore, expression of the \( \alpha_2(I) \) gene in chondrocytes and BrdUrd-shifted cells is regulated by choice of the transcription start site.

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sequencing gels were as described (22). The autoradiograms from this procedure and from the run-on assays (see below) were quantitated with a laser beam scanning densitometer. In all cases, multiple exposures were scanned to ensure that the absorbance fell within the linear range of absorbance versus radioactivity.

Run-on Transcription Assays—After chilling the cells on ice, nuclei were isolated by the procedure of Konicek and Emerson (23), performed at 4°C. Nuclei, after storage at -80°C, were assayed in exposures were scanned to ensure that the absorbance fell within the linear range of absorbance versus radioactivity.

incubation was for 20 min at 27°C. The 3P-labeled run-on transcripts were adjusted to 2-5 × 10⁶ cpm/ml in hybridization buffer: 50% formamide, 50 mM sodium phosphate, pH 7.4, 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 1 mM EDTA, 0.2% sodium dodeyl sulfate, and 1 × Denhardt’s solution (25). Hybridization was performed at 42°C for 48 h in the presence of 2 µg of each cDNA, excised and purified free from vector sequences. The DNA was dot-blot-blotted to Genescreen (Du Pont-New England Nuclear). This level of DNA was found to be in excess relative to the RNA present in run-on reactions (data not shown). Washing and RNase treatment of the filters was essentially as described by Greenberg (26).

Polymerase Chain Reaction—The procedure was basically that of Conboy et al. (27). First strand cDNA synthesis was carried out on total cellular RNA with Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) in the presence of an oligodeoxynucleotide primer containing 18 nt identical with the antisense strand of α(I) exon 3. Duplicate first strand cDNA samples were then amplified with AmpliTaq (Perkin-Elmer) with the same 3’ primer as above but with either a 5’ primer identical with the first 21 nt of exon 1 or a 5’ primer identical with nt +9 to +28 of exon A. Twenty-five PCR cycles were performed with a DNA Thermal Cycler (Perkin-Elmer Cetus) at 94°C for 1 min, at 40°C for 1 min, and at 72°C for 3 min. The primers were supplied by Oligos Etc., Inc. PCR products were analyzed on agarose gels consisting of 3% NuSieve (FMC Corp.) and 1% ultrapure agarose (Bethesda Research Laboratories), and the gels were stained with ethidium bromide.

RESULTS

Transcription Rates of Type I and Type II Collagen Genes in Untreated and BrdUrd-treated Chondrocytes—The amount of radioactive type I and II collagen transcripts synthesized in nuclear run-on assays by nuclei from untreated or BrdUrd-treated chondrocytes was measured by hybridization of the transcripts to immobilized cDNAs and by densitometry of autoradiograms (Fig. 2). The hybridization to nonspecific DNA (pUC) was subtracted, and the net hybridization was corrected for the relative extent of 27 S rRNA synthesis by the two types of nuclei prior to calculating the relative transcription rates for the collagen genes in untreated chondrocytes as compared to BrdUrd-treated chondrocytes. The radioactive collagen transcripts were shown to be produced by RNA polymerase II, since α-amanitin at 2 µg/ml completely abolished their synthesis (data not shown).

These measurements showed that the α(I) gene is transcribed at approximately the same rate in chondrocyte nuclei as in the nuclei from BrdUrd-treated cells. The α(I) transcription rate in untreated chondrocytes was found to be 0.86 times the rate in BrdUrd-treated chondrocytes in the experiment shown in Fig. 2, after normalizing the radioactivity incorporated into the α(I) transcripts to the radioactivity incorporated into 27 S ribosomal RNA by the nuclei from the two types of cells. The α(I) cDNA probe used in this experiment, excised from the plasmid pUCG54 (15), contains a long collagensous sequence, 602 nucleotides coding for the triple helical region, and 572 nucleotides coding for the COOH-terminal propeptide. Similar results were obtained in subsequent experiments with the 344-bp probe that was shown to be specific for α(I) sequences (Fig. 1); a representative example is shown in the bottom horizontal line of Fig. 2. In this experiment, the relative rate of α(I) transcription by chondrocyte nuclei was found to be 1.06 times the transcription

FIG. 1. Test of specificity of the three collagen cDNA probes with in vitro-transcribed “sense-strand” collagen RNAs. The sense-strand RNAs are identical in sequence with the corresponding regions of the mRNAs and were synthesized by in vitro transcription of cloned collagen cDNA templates with T3 or T7 RNA polymerase (see “Materials and Methods”). A, the locations of the synthetic RNA sequences (lines above solid boxes) are shown relative to collagen mRNA (top line) (15, 18). The cDNA probes used in the nuclear run-on transcription experiments, and as templates for the RNA probes in the RNase protection experiments, are shown as solid rectangles. B, the cDNA probes were excised from the parent plasmids and electrophoresed on agarose gels prior to transfer to nitrocellulose by blotting. Lanes 1–3 of each gel contained, respectively, the α(1)I, α(1)II, and type II cDNA probes, which have sizes, respectively, of 344, 236, and 215 bp (the shorter α(I)I probe was used). Each blot was hybridized with the 32P-labeled in vitro-synthesized mRNA sequences diagrammed in A, as indicated below each panel. The conditions for hybridization and washing the blots were exactly the same as those used in the nuclear run-on transcription assay. The autoradiograms were overexposed to allow for detection of weak cross-hybridization.

FIG. 2. Nuclear run-on assays of transcription rates of type I and II collagen genes in untreated and in BrdUrd-treated chondrocytes. The cDNA probes used for α2(I) and type II transcripts were, respectively, the 236- and 215-bp cDNAs shown in Fig. 1A; the α(1)I cDNA used in the top horizontal lane was from pUCG54 as described in the text, and in the bottom horizontal lane from a different experiment, was the 344-bp probe shown in Fig. 1A. DNA from the plasmid vector pUC19 was used as a control for hybridization of transcripts to nonspecific DNA. The hybridization of 27 S ribosomal transcripts to the 27 S ribosomal cDNA provided a measure of the relative transcriptional activity of each sample of nuclei.
rate of nuclei from BrdUrd-treated chondrocytes. Additional experiments with each of these probes and with the 169-bp α1(I) probe (Fig. 1A) also showed that the transcription rate of the α1(I) gene is approximately the same in untreated and BrdUrd-treated chondrocytes. The variability in the values obtained in this assay, however, prevents us from determining a precise ratio; we can only conclude that the difference in transcription rates is relatively modest, not differing by more than a factor of 2 in either direction.

The relative transcription rate of the α2(I) gene in untreated chondrocytes was found to be 0.98 times the transcription rate in BrdUrd-treated chondrocytes in the experiment shown in Fig. 2, after correcting for the relative rates of ribosomal RNA synthesis. In this experiment, the 236-bp α2(I) cDNA of proven specificity (Fig. 1) was used. Additional assays with this cDNA, as well as earlier assays with the large 2,500-bp α2(I) cDNA insert from the plasmid pCG45, also demonstrated that untreated chondrocyte nuclei synthesized α2(I) transcripts at approximately the same rate as the nuclei of BrdUrd-shifted cells. Again, the variability in this assay does not allow us to exclude a 2-fold difference in these rates.

The nuclear run-on measurements of the transcription rate of the type II collagen gene gave an experimental result markedly different from that obtained for the type I genes. The type II gene showed a marked and consistent decrease in transcription rate in the nuclei from BrdUrd-treated chondrocytes. In Fig. 2, the transcription rate of the type II gene in the BrdUrd-treated chondrocytes is below the detectable level, but in other experiments the rate varied from 0 to 0.23 times the rate in untreated chondrocytes. Since this variability probably arises in this case not only from the variability of the assay, but also from variability in the completeness of the BrdUrd-induced shift at the time of preparing the nuclei, the exact ratio is probably not very significant. In the case of this gene, however, the nuclear run-on measurements lead to the important conclusion that the turn-off of expression of the type II collagen gene in BrdUrd-treated cells is achieved primarily by control at the level of transcription.

Degradation Rates of Type I and II Collagen mRNAs in Untreated and BrdUrd-treated Chondrocytes—To assess the contribution of mRNA stability in the BrdUrd-induced changes in the levels of the type I and II collagen RNAs (11), the degradation rates were measured for each of these RNAs in untreated chondrocytes and in BrdUrd-treated chondrocytes. The level of each RNA was measured by RNase protection of RNA probes synthesized from the gene-specific cDNA clones shown in Fig. 1 (the shorter α1(I) cDNA was used). The degradation rates were obtained by observing the decrease in the concentration of each RNA at various time intervals after the addition of sufficient actinomycin D to inhibit further mRNA synthesis.

The results of these assays are shown as representative autoradiograms in Fig. 3 and as decay curves in Fig. 4, where the results from scanning the autoradiograms from two or more experiments are plotted. Since the α1(I) gene is transcribed as rapidly in chondrocytes as in BrdUrd-shifted cells, but its steady state level in chondrocytes is only 1.5% that of shifted cells (see below), the expectation was that its degradation rate must be much faster in chondrocytes than in shifted cells. The experimental results, however (Figs. 3, A and C, and 4B), show no significant change in the degradation rate of α1(I) RNA in chondrocytes, with an approximate half-life of 12 h in both types of cells. The fibroblastic decay rate, along with the low levels of this RNA, suggests that this RNA is derived from the perichondrial fibroblasts that contaminate these chondrocyte cultures. The variable levels of α1(I) mRNA present in different chondrocyte RNA preparations support the conclusions that this RNA is not an authentic chondrocyte product. From observations in other systems (see “Discussion”) it is likely that the turn-over of α1(I) transcripts is extremely rapid in chondrocytes and not measurable by the method employed here.

The α2(I) RNA appears to be degraded more rapidly in untreated chondrocytes (Figs. 3, B and C, and 4C), t1/2 = 5.2 h, than in BrdUrd-treated chondrocytes, t1/2 = 10.4 h. While this is a relatively modest change in stability, it may account for the higher steady state level of α2(I) RNA in BrdUrd-treated chondrocytes. Since this RNA has a different 5' end in chondrocytes than in BrdUrd-treated chondrocytes (see below), it is not surprising that the two different RNA species might have different decay rates. Because of the relatively high level of the α2(I) RNA in untreated chondrocytes, the low level of contamination of these cultures by fibroblasts (5% maximum) would introduce a maximum contamination by fibroblast α2(I) RNA of 10%.

The half-life of type II collagen RNA after BrdUrd treatment is not significantly different from its half-life in untreated chondrocytes (Figs. 3, D and E and 4A), with an average value of approximately 15 h. The decrease in expression of this gene in BrdUrd-treated chondrocytes is achieved, therefore, primarily by decreasing its transcription rate, rather than by changes in the stability of the mRNA.

Relative Levels of the Type I and Type II Collagen mRNAs in Chondrocytes and BrdUrd-treated Chondrocytes—The RNase protection assays used to measure decay rates provided, as a by-product, a very sensitive and specific measurement of the levels of these RNAs in the time zero samples, taken before the addition of actinomycin D. These measurements confirmed our earlier measurements that used RNA dot-blots and Northern blots (11). The level of α1(I) RNA in total cellular RNA is very low in chondrocytes relative to BrdUrd-shifted cells (1.5 ± 1.3%). The level of α2(I) RNA in chondrocytes, relative to shifted cells, is again found to be surprisingly high (28 ± 7.5%). The marked decrease seen in the level of type II RNA in BrdUrd-treated cells compared with untreated cells (5% vs. 6%) is also consistent with the earlier measurements, although in the earlier measurements the type II mRNA was undetectable in shifted cells. It is possible that the RNA dot-blot and Northern blot procedures used previously, unlike the more sensitive RNase protection assay, would not have detected the low level of type II RNA in shifted cells, but it is also likely that, due to variation in the time course of the BrdUrd-induced shift in different experiments, the shift may not have gone to completion in these cultures.

The α2(I) RNA in These Chondrocytes Differs in Its 5'-End from the Fibroblast-type α2(I) mRNA—It was important to know whether the α2(I) in these chondrocytes contained the altered 5'-end previously reported by Adams and co-workers (12, 13) to be present in embryonic chick chondrocytes, since such an RNA would not be expected to code for collagen α2(I) chains (13). The presence of this altered α2(I) RNA in these chondrocytes would indicate that a change in the location of the transcription start site was a major regulatory mechanism in the turn-on of the synthesis of the α2(I) collagen chains in BrdUrd-treated cells. Since the presence of this altered RNA is influenced by the culture conditions (12) and might also be influenced by the age of the sternum from which the chondrocytes were obtained (see, for example, Ref. 28), we wished to see whether our cultured sternal chondrocytes contained

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this α2(I) RNA species. For this purpose, the PCR technique was used to detect specifically both kinds of α2(I) RNA, the fibroblast form containing the fibroblast exons 1 and 2 spliced to exon 3, and the chondrocyte form containing the chondrocyte exon A within intron 2 (13). BrdUrd treatment, however, shifts the transcription start site utilized by these chondrocytes to the fibroblastic site, at the start of exon 1. Similar conclusions have been reached by means of RNase protection experiments.

**DISCUSSION**

The studies reported here support the conclusion that the switch that turns off the expression of the type II collagen gene during the BrdUrd-induced shift is acting primarily at the transcriptional level. The nuclear run-on transcription assays show that the type II gene is transcribed to a much lesser extent in BrdUrd-shifted cells than in untreated cells, while the degradation rate of type II mRNA shows little or no change. The conclusion that the type II gene is controlled primarily at the transcriptional level in this system is consistent with our earlier observation that the level of type II mRNA decreases to very low levels in both the nucleus and cytoplasm of chondrocytes exposed to BrdUrd for 6 to 8 days.

**Fig. 3.** RNase protection assay of the type I and II collagen mRNAs remaining after incubation of untreated or BrdUrd-treated chondrocytes in the presence of actinomycin D. Total cellular RNA (20 µg in A, B, and E, 2 µg in C and D) was assayed for α1(I), α2(I), and type II RNA by densitometry of the protected fragments shown in the autoradiograms above. Lanes 0 through 8 indicate the hours of exposure of the cells to 5 µg/ml actinomycin D; lane M contains a size marker of 32P-labeled MspI-digested pBR322; lane P contains unincubated 32P-labeled probes alone; and lane C contains 32P-labeled probes treated exactly as the samples in lanes 0–8, except that tRNA was substituted for the cellular RNA during the hybridization. Due to transcribed vector sequences, the probes are larger than their protected sequences, with sizes of 220, 262, and 249 bp, respectively, for the α1(I), α2(I) and type II probes. In A–C, both type I probes were used to assay α1(I) and α2(I) RNAs simultaneously. The autoradiogram in B was overexposed in A to allow measurements of α1(I) RNA. The apparently lower level of RNA in the 0-time samples in C and D is due to experimental error, as is the anomalously high levels of both type I RNAs in the 2-h sample in A and B, since these results were not seen in other experiments (compare Fig. 4). In D, lane P, the band visible near 215 bp is the 220-bp α1(I) probe which was added, along with the 249-bp type II probe, to this lane.
Levels of Regulation in BrdUrd-induced Switch from Collagen Type II to I

Fig. 4. Decay curves of type I and II collagen mRNAs in untreated (○—○) and BrdUrd-treated (●—●) chondrocytes. After the indicated time of exposure to actinomycin D, the amount of each of the three collagen mRNAs was measured by densitometry of autoradiograms (see Fig. 3). The values with the average deviation from the mean are plotted on a logarithmic scale as the percent of the time zero value, and the curve is drawn by the method of least squares. The values for a1(I) and type II RNAs are from two experiments and for a2(I) from three experiments. Regression analysis shows no significant differences between the decay rates of the type II and a1(I) mRNAs in the two-cell type (t test for difference in regression slopes: p = 0.29 and 0.12, respectively). The a2(I) RNA decays, however, significantly faster in untreated chondrocytes (p = 0.037).

The control of type II collagen gene expression has also been observed to occur primarily at the transcriptional level in a different experimental system. In this system, cultures of adherent dedifferentiated chondrocytes, derived from chick embryonic tibia, are induced to undergo a phenotypic shift in the reverse direction from that studied here, by transfer to suspension culture. As these cultures shift from the synthesis of type I to type II collagen, the increase in type II mRNA concentration is accompanied by a corresponding increase in the transcription rate of this gene (29, 30). The further analysis of the mechanism that regulates the transcription rates of the type II collagen gene, such as the identification of the DNA sequences and the trans-acting transcription factors that are involved, is likely to require the isolation of presently unavailable clones of the 5' region and 5'-flanking sequences of the chick type II collagen gene.

Although the untreated chondrocytes do not produce type I collagen polypeptides, they appear to transcribe the type I genes as rapidly as BrdUrd-treated chondrocytes, which do produce type I collagen. This conclusion is based on the similar transcription rates observed in nuclear run-on assays with nuclei from untreated or BrdUrd-shifted chondrocytes. Two observations, in agreement with the experience of others (23), suggest that this nuclear run-on system is accurately reflecting the activity of the nuclei in intact cells rather than transcribing DNA indiscriminately. First, the marked decrease in the rate of transcription of the collagen type II gene in the nuclei from BrdUrd-shifted cells, relative to the rate in untreated cells, while little change is occurring in the relative rate of transcription of the type I genes, indicates that a specific gene can be selectively transcribed by this system. Second, the nuclei from chicken intestinal epithelial cells which would be expected to synthesize little or no type I or II collagen mRNAs, transcribed these three genes at rates that were 4 to 8% of the rates observed in the nuclei of untreated chondrocytes (data not shown).

The turn-on of type I collagen gene expression in these
BrdUrd-treated chondrocytes, without a corresponding increase in the transcription rate of these genes, appears to represent a different mode of control than that which occurs in the case of the α1(I) gene (the α2(I) gene was not studied) in the dedifferentiated tibial chondrocytes, referred to above (29, 30). In this system, as the adherent chondrocytes are induced to shift from type I to II collagen synthesis by transfer to suspension culture, the decrease in α1(I) mRNA levels is achieved by a corresponding decrease in the transcription rate of this gene. The explanation for this difference in the regulation of expression of the α1(I) gene in these two systems is unknown but could be related to the different tissue origin of the chondrocytes (14-day sternum versus stage 28–30 tibia) or to differences in the treatment of the cells. The shift from type II to type I collagen synthesis in the present report is induced by BrdUrd treatment of chondrocytes that were selected as “floaters” after 3 days of culture, whereas the shift from type I to type II collagen synthesis in tibial chondrocytes was induced by transfer to suspension culture of chondrocytes that were first differentiated by 3 weeks of culture as adherent cells.

Although the rate of α1(I) gene transcription is not drastically decreased in the untreated chondrocytes relative to the rate in BrdUrd-shifted chondrocytes, the steady state level of the α1(I) RNA in untreated chondrocytes is very low, less than 2% of its level in BrdUrd-shifted cells. This finding is consistent with the observations of others that a low level of α1(I) mRNA is present in sternal chondrocytes (31, 32). Since the transcription rate of the α1(I) gene is comparable in both cell types, it is necessary to postulate that the rate of degradation of this mRNA is much faster in chondrocytes than in shifted cells, in order to explain its low level in chondrocytes. The observed rate of decay of the α1(I) RNA in untreated chondrocytes was, however, identical with its decay rate in BrdUrd-shifted cells. For the reasons noted under “Results,” this RNA is likely to be derived from the low level of fibroblasts that contaminate these chondrocyte cultures. The explanation we favor for the failure to detect a rapidly decaying α1(I) RNA species in untreated chondrocytes is that the pre-RNA α1(I) transcripts are turning over so rapidly in the nuclei of these chondrocytes that they escape detection in our assay.

Regulation of steady state mRNA levels by changes in the efficiency of nuclear processing or export appears to be rare relative to regulation of rates of transcription or of cytoplasmic mRNA degradation. However, a few examples demonstrate exceptions to this pattern. In most of these cases, the evidence is indirect and similar to that reported here for the α1(I) collagen mRNA: a change in mRNA level is observed in two or more situations without a change in either the transcription rate of the gene, or the decay rate of the processed mature mRNA (see for examples, Refs. 33–36). Direct evidence for the regulation of cytoplasmic mRNA levels by changes in the efficiency of nuclear processing or export, relative to the efficiency of nuclear decay, has been much harder to obtain (see for example, Refs. 37–40).

The expression of the collagen α2(I) gene is, like the α1(I) gene, not regulated in these chondrocytes by major changes in its rate of transcription. Unlike the α1(I) RNA, the α2(I) RNA is present in relatively high levels in these chondrocytes, at approximately one-third its level in BrdUrd-shifted chondrocytes. The more rapid decay of the α2(I) RNA in the untreated chondrocytes could account for this decrease. These conclusions were reached with the aid of cDNAs complementary to the 3′ end of the mRNA. The α2(I) RNA present in these untreated chondrocytes was found, however, by PCR technology, to differ at its 5′ end from the fibroblast form of α2(I), in agreement with previous reports of such altered α2(I) RNA in embryonic chick chondrocytes (12, 13). This altered RNA is missing exons 1 and 2 (see diagram in Fig. 5) and the new first exon, exon A, is reported to be spliced to the usual fibroblastic exon 3 in a noncollagenous reading frame (13). The presence of this altered form of chondrocyte α2(I) RNA therefore provides an explanation for our previous observation that α2(I) collagen chains were not synthesized by these chondrocytes, in spite of the presence of α2(I) RNA, as assayed with 3′ probes, in the cytoplasm in polyadenylated form (11). The regulation of the α2(I) gene in chondrocytes appears, therefore, to occur primarily by a change in location of the transcription start site. The same regulatory mechanism, operating in the reverse direction to turn off the expression of the α2(I) gene, appears to occur during the switch from type I to type II synthesis as precursor mesenchyme cells differentiate into chondrocytes (13). The physiological role of the altered chondrocyte transcript as well as the mechanism that effects the change in the transcription start site at present unknown. Another example of a tissue-specific difference in the transcription start site of a chick collagen gene is provided by the recent report (41) that the transcription start site of the α1(IX) collagen gene in corneas is in intron 6 of the cartilage gene, 20 kb 3′ to its start site in cartilage. In this case, however, the two reading frames are in phase, so that the cornea transcript codes for a shortened version of an α1(IX) collagen, rather than for a noncollagenous protein, as in the case of the cartilage version of the α2(I) RNA.

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