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Control of Types I and II Collagen and Fibronectin Gene Expression in Chondrocytes Delineated by Viral Transformation

EILEEN S. ALLEBACH,[1] DAVID BOETTIGER,[2] MAURIZIO PACIFICI,[3] AND SHERRILL L. ADAMS[1]*

Departments of Human Genetics,[1] Microbiology,[2] and Anatomy,[3] University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

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We have analyzed the effects of transformation by Rous sarcoma virus on expression of types I and II collagen and fibronectin genes in vertebral chondrocytes and compared them with expression of these genes in skin fibroblasts. Transformed chondrocytes display a dramatically decreased amount of type II collagen RNA, which can account fully for the decreased synthetic rate of this protein. Paradoxically, these cells also display greatly increased amounts of type I collagen RNAs, which are translated efficiently in vitro, but not in the intact cells. We show here that the type I collagen RNAs in transformed chondrocytes are nearly indistinguishable from those found in skin fibroblasts, and that they clearly differ from the type I collagen RNAs found in normal chondrocytes. Transformed chondrocytes also display an increased amount of fibronectin RNA, which can account fully for the increased synthetic rate of this protein. Thus, the effects of transformation by Rous sarcoma virus on type I collagen and fibronectin RNAs in chondrocytes are the opposite of those observed in fibroblasts, which display decreased amounts of these three RNAs. These data indicate that the effects of transformation on the genes encoding type I collagen and fibronectin must be modulated by host cell-specific factors. They also imply that the types I and II collagen genes may be regulated by different mechanisms, the type I genes being controlled at both transcriptional and posttranscriptional levels, and the type II gene being controlled primarily at the transcriptional level.

Transformation of differentiated cells by tumor viruses has provided a useful tool for analyzing the mechanisms of cellular gene expression. This has been particularly true for the genes encoding extracellular matrix proteins, whose expression is decreased by transformation with certain viruses. For example, transformation of chicken embryo fibroblasts by Rous sarcoma virus (RSV) results in reduced synthesis of type I collagen (10, 20, 25, 45–47) and, in some cases, fibronectin (36, 39). Similarly, transformation of chicken vertebral chondrocytes by RSV results in decreased synthesis of type II collagen and the major cartilage proteoglycan (2, 18, 37, 38). The reduced rates of synthesis of type I collagen and fibronectin in transformed fibroblasts reflect decreased amounts of the corresponding RNAs (1, 3, 15, 19, 43, 45, 46, 51) due to decreased rates of transcription of these genes (46, 53).

Recent experiments from our laboratories have suggested that the mechanisms controlling the synthesis of some extracellular matrix proteins in chondrocytes may be more complex than the transcriptional controls that apparently predominate in fibroblasts. Chicken vertebral chondrocytes normally synthesize type II, but not type I, collagen, and synthesize only a small amount of fibronectin. Upon transformation by RSV, these cells display a decreased amount of type II collagen, synthesize a large amount of fibronectin (2, 37, 66), and accumulate type I collagen RNAs that are translated efficiently in vitro, but are apparently not translated in the intact cells (2).

To better understand the mechanisms controlling types I and II collagen and fibronectin gene expression, we have examined both quantitatively and qualitatively the effects of RSV transformation on the RNAs encoded by these genes in chondrocytes. These results have been compared with the effects of RSV transformation on type I collagen and fibronectin gene expression in skin fibroblasts. The experiments we describe below indicate that the decrease in type II collagen synthesis in transformed chondrocytes is a direct reflection of dramatically decreased RNA levels, and that the increase in fibronectin synthesis corresponds to increased RNA levels. The type I collagen RNAs accumulate to high levels, and their electrophoretic mobilities are altered from the RNAs of unique size found in normal chondrocytes to RNAs nearly identical to those found in normal fibroblasts.

These data demonstrate that transformation by RSV has opposite effects on the expression of type I collagen and fibronectin genes in chondrocytes and fibroblasts and imply that the action of *src* is modulated by host cell factors that differ between these cell types. They also imply that the types I and II collagen genes may be regulated by different mechanisms. The genes encoding type I collagen, a protein which is synthesized by many cell types, appear to be regulated at both transcriptional and posttranscriptional levels. In contrast, the type II collagen gene, which is expressed only by a very limited number of terminally differentiated cell types, appears to be regulated primarily at the level of transcription.

MATERIALS AND METHODS

Cells and viruses. All cells were derived from pathogen-free chicken embryos (SPAFAS, Inc., Norwich, Conn.). The preparation and infection of primary chondrocytes derived from the ventral portion of the vertebral column have been described in detail previously (7, 38). Cells were infected with RSV, either the wild-type strain Prague A (wt-RSV) or the conditional mutant ts-LA24A (ts-RSV) (60), at a multiplicity of 1 to 3 focus-forming units per cell. Cells infected with the mutant virus were allowed to grow for two or three passages until they were fully infected, at which time they were trypsinized and replated on bacterial culture dishes, so that the cells remained in suspension (2). Half of the cultures

* Corresponding author.

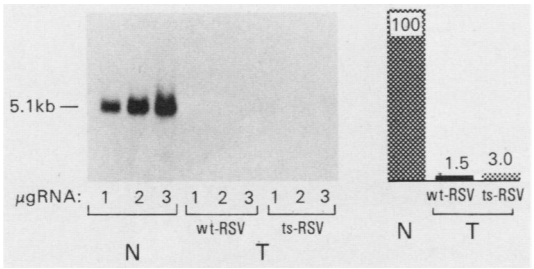

FIG. 1. Northern hybridization analysis of type II collagen RNAs. Total cellular RNAs isolated from uninfected chondrocytes and from wt-RSV- and ts-RSV-transformed chondrocytes were denatured with formamide-formaldehyde, separated by electrophoresis on a 1% agarose gel, transferred to Gene Screen, and hybridized at high stringency (16) with the type II collagen cDNA pCgII-12 (28). Each lane was scanned with a densitometer, and the areas under the peaks were determined. The histogram displays the amounts of the type II collagen RNA in transformed cells (T) relative to the amount in normal chondrocytes (N). kb, Kilobase.

were immediately shifted to 41°C, and the other half remained at 36°C. Cells infected with ts-RSV and grown at 41°C display a normal phenotype and are referred to as phenotypically normal; those grown at 36°C display a transformed phenotype, and are referred to as transformed. The cultures were harvested 3 days after the temperature shift.

Primary skin fibroblasts were prepared from 12-day-old embryos as described previously (16) and allowed to attach to the substratum for 2 to 4 h. Infections with the virus strains described above were carried out at a multiplicity of 0.3 to 0.5 focus-forming units per cell. Cells were allowed to grow for 1 or 2 passages until they were fully infected, at which time they were trypsinized and replated. Temperature shifts were performed as described above for chondrocytes.

Northern hybridization. Total cellular RNAs (3, 8, 52) were denatured either by glyoxalation (31) or by treatment with formaldehyde and formamide (23), as indicated in the figure legends; several concentrations of each RNA were analyzed to insure linearity of hybridization with RNA concentration. In addition, *Hin*dIII fragments of bacteriophage lambda DNA were included as size markers on every gel. Samples were electrophoresed on 1% agarose gels and transferred to Gene Screen (New England Nuclear Corp., Boston, Mass.) as recommended by the manufacturer. Blots were prehybridized, hybridized, and washed with either the high- or low-stringency conditions described previously (16), as indicated in the figure legends.

The following probes were used in the Northern hybridizations: pFN600, a 600-base-pair fibronectin cDNA (14); pCOL3, an 800-base-pair α1(I) collagen cDNA (64); pCg45, a 2,500-base-pair α2(I) collagen cDNA (24); pCgII-12, a 740-base-pair α1(II) collagen cDNA (28); and pHrB, a human 18S ribosomal DNA (59). The plasmids pFN600, pCg45, pCgII-12, and pHrB were generous gifts of K. Yamada and B. de Crombrugghe, H. Boedtker, L. Lukens, and J. Sylvester, respectively. Intact plasmid DNAs were nick translated (42), and 5×10^6 dpm of the collagen cDNAs, 10^7 dpm of the fibronectin cDNA, 10^6 dpm of the 18S ribosomal DNA, and 2×10^4 dpm of bacteriophage lambda DNA were used in the hybridizations.

Kodak X-Omat XAR-5 film was exposed for various lengths of time at −80°C with a Dupont Cronex Lightning Plus intensifying screen. The films were scanned and quantitated as described above. Each value was expressed as a percent of the highest synthesis for that cell type; when necessary, data were normalized for variations in RNA concentration by using the values obtained by 18S ribosomal DNA hybridization.

RESULTS

Effect of viral transformation on type II collagen RNAs. RNAs from normal and transformed chondrocytes were subjected to Northern hybridization analysis with ^{32}P-labeled pCgII-12, a cloned type II collagen cDNA (28) (Fig. 1). A very prominent RNA of 5,100 bases was detected in normal chondrocytes, as described previously (16, 28, 57). Transformed chondrocytes displayed reductions in this RNA species to 1.5% (wt-RSV-transformed cells) and 3% (ts-RSV-transformed cells) of normal levels, consistent with the dramatic reduction in the rate of type II collagen synthesis in these cells (2). The type II collagen probe did not hybridize to any RNAs in normal or transformed skin or tendon fibroblasts (data not shown) (16).

Effect of viral transformation on amounts of type I collagen RNAs. RNAs from normal and transformed chondrocytes were also analyzed by Northern hybridization with ^{32}P-labeled pCOL3, a cloned α1(I) collagen cDNA (64) (Fig. 2A). In phenotypically normal chondrocytes (infected with ts-RSV and grown at 41°C), this probe identified a small amount of RNA that was comparable to that found in uninfected chondrocytes (16). In transformed chondrocytes (infected with ts-RSV and grown at 36°C), the amount of hybridizing RNA increased 5.3-fold to a level similar to that found in phenotypically normal fibroblasts.

The same RNAs from normal and transformed chondro-

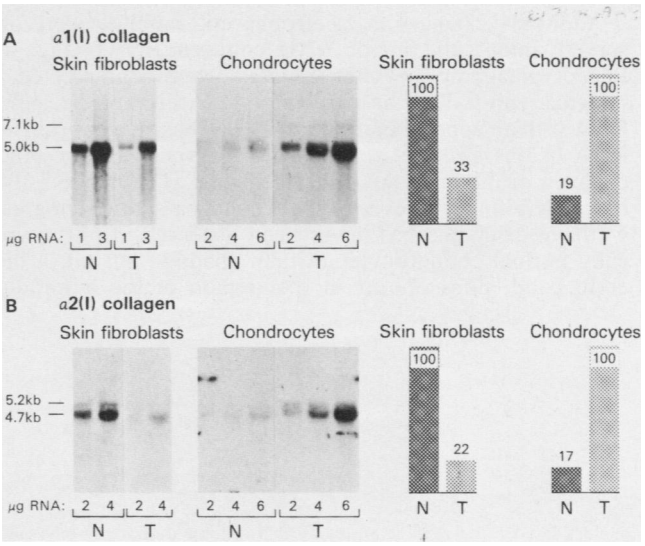

FIG. 2. Northern hybridization analysis of type I collagen RNAs. Total cellular RNAs from normal (N) and transformed (T) skin fibroblasts and vertebral chondrocytes were denatured by glyoxalation; denatured RNAs were then separated by electrophoresis on 1% agarose gels and transferred to Gene Screen. In this experiment, all cells were infected with ts-RSV and grown at the nonpermissive (N) or permissive (T) temperature for transformation. The RNAs were hybridized with (A) the α1(I) collagen cDNA pCOL3 (64) and (B) the α2(I) collagen cDNA pCg45 (24). Chondrocyte RNAs in panel A were hybridized at high stringency (16); other RNAs were hybridized at low stringency. Band intensities were determined as described in the legend to Fig. 1 and are displayed in the histogram as a percentage of the highest value for that cell type. All values were normalized to 18S rRNA levels. kb, Kilobase.

cytes were also analyzed by hybridization with ^{32}P-labeled pCg45, a cloned α2(I) collagen cDNA (24) (Fig. 2B). In phenotypically normal chondrocytes, this probe identified two RNAs that were similar in amount to those reported for uninfected chondrocytes (16). The transformed chondrocytes displayed a 5.9-fold increase in the amounts of these RNAs; this is nearly identical to the increase in α1(I) collagen RNAs after transformation (Fig. 2A).

Thus transformation of chondrocytes by RSV resulted in a coordinate increase in RNAs encoding the two subunits of type I collagen. This was precisely the opposite of its effect on expression of the type I collagen genes in skin fibroblasts (shown for comparison in Fig. 2), in which the amount of α1(I) collagen RNA was decreased 3.3-fold and the amount of α2(I) collagen RNA was decreased 4.5-fold.

Effects of viral transformation on electrophoretic mobilities of α1(I) collagen RNAs. We have previously shown that the major α1(I) collagen RNA in normal chondrocytes has an estimated size of 5,600 bases, instead of the usual 5,000-base RNA found in most type I collagen-synthesizing cells (16). To determine whether transformation of chondrocytes by RSV altered the electrophoretic mobilities of the α1(I) collagen RNAs, we analyzed these RNAs in more detail, using gels twice as long as those shown in other experiments (Fig. 3). In this experiment, uninfected chondrocytes displayed the expected 5,600-base RNA. In contrast, chondrocytes transformed with wt-RSV displayed a hybridization pattern that appeared identical to that observed in normal and transformed fibroblasts, with a major RNA of about 5,000 bases. The use of the longer gel has allowed us to resolve this major RNA into two species, with estimated sizes of 5,000 and 5,200 bases in transformed chondrocytes, as well as in normal and transformed fibroblasts.

Additional changes in electrophoretic mobility were observed among the minor α1(I) collagen RNAs (Fig. 4). Phenotypically normal chondrocytes, whether uninfected or infected with ts-RSV and grown at 41°C, displayed a minor RNA with an approximate size of 10,000 bases; a hybridizing RNA of this size has never been observed with any other collagen probe or in any other cell type. The minor 7,100-base RNA found in every type I collagen-synthesizing cell we have examined has never been observed in phenotypically normal chondrocytes (a faint shadow, but not a discrete band, does appear in that region of the autoradiograph). In contrast, transformed chondrocytes displayed a pattern nearly identical to that observed in fibroblasts, with a minor 7,100-base RNA and no 10,000-base RNA. In addition, a higher-molecular-weight RNA of 18,000 to 20,000 bases was present. The heart fibroblast RNA used for a control in this experiment has been overexposed, to illustrate that neither the 10,000- nor the 18,000- to 20,000-base RNAs can be detected in these cells.

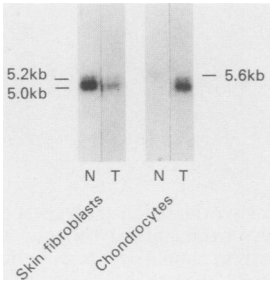

FIG. 3. Effects of transformation on the sizes of the major α1(I) collagen RNAs. Total cellular RNAs isolated from skin fibroblasts infected with ts-RSV and grown at 41°C (N) and 36°C (T) and from primary chondrocytes (N) and chondrocytes infected with wt-RSV (T) were denatured with formaldehyde-formamide, separated by electrophoresis on a 20-cm-long agarose gel, transferred to Gene Screen, and hybridized with the α1(I) collagen cDNA pCOL3 (64) at high stringency (16). The resolution of the major hybridizing species into two RNAs of 5,000 and 5,200 bases can be clearly seen. kb, Kilobase.

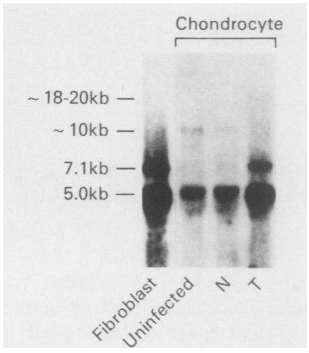

FIG. 4. Effects of transformation on the sizes of the minor α1(I) collagen RNAs. Total cellular RNAs (5 μg) isolated from heart fibroblasts, primary chondrocytes, and ts-RSV-infected chondrocytes at 41 and 36°C were denatured by glyoxalation, separated by electrophoresis on a 1% agarose gels, transferred to Gene Screen, and hybridized at low stringency with the α1(I) collagen cDNA pCOL3 (64). The minor 10,000-base RNA in normal chondrocytes and 18,000- and 20,000-base RNA in transformed chondrocytes can be clearly seen. kb, Kilobase.

Correlation of changes in fibronectin synthesis with altered RNA levels. Quantitative studies of fibronectin RNAs in normal and transformed vertebral chondrocytes were also undertaken to determine whether altered steady-state RNA levels were responsible for the altered rates of fibronectin synthesis (Fig. 5). Northern transfers of RNAs from phenotypically normal chondrocytes displayed a low level of the 8,800-base fibronectin RNA, detected by hybridization with the ^{32}P-labeled fibronectin cDNA clone, pFN600 (14). Transformed chondrocytes showed a fourfold increase in fibronectin RNAs, to a level roughly comparable to that found in phenotypically normal skin fibroblasts. In contrast, trans-

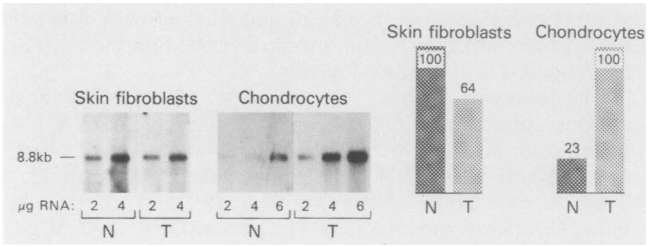

FIG. 5. Northern hybridization analysis of fibronectin RNAs. Total cellular RNAs from normal (N) and transformed (T) skin fibroblasts and vertebral chondrocytes were denatured by glyoxalation, separated by electrophoresis on 1% agarose gels, transferred to Gene Screen, and hybridized with the fibronectin cDNA pFN600 (14). In this experiment, all cells were infected with ts-RSV and grown at the nonpermissive (N) or permissive (T) temperature for transformation. Band intensities were determined as described in the legend to Fig. 1, and areas were plotted against amount of RNA. The best-fitting lines were drawn through the points, and the slopes of the lines were calculated. These values are displayed in the histogram as percentages of the highest values for the cell types. kb, Kilobase.

formed skin fibroblasts displayed the expected decrease in steady-state fibronectin RNAs (Fig. 5). Thus viral transformation also has opposite effects on fibronectin RNA levels in chondrocytes and skin fibroblasts.

DISCUSSION

Modulation of transformation effects by host cell factors. The transformation of several kinds of terminally differentiated cells by RSV results in the suppression of synthesis of their characteristic products (2, 6, 13, 37, 38). This suppression could be explained by postulating that the viral *src* gene product acts on a common pathway that controls the expression of all such differentiated products. The data presented here are not inconsistent with this hypothesis, but indicate that the mechanisms may be more complex than previously supposed. The activation of *src* has opposite effects on the expression of type I collagen and fibronectin genes in vertebral chondrocytes and skin fibroblasts. Their expression is enhanced in chondrocytes, which normally synthesize these proteins only at low levels, if at all, and suppressed in fibroblasts, which normally synthesize these proteins at high levels. Thus it appears that the effects of viral transformation on genes encoding differentiated cell products depend not only on the expression of the viral *src* gene, but also on host cell factors specific for each cell type which may modify the effects of *src*.

Processing of α1(I) collagen RNAs. The posttranscriptional processing of the α1(I) collagen RNAs appears to be more complex than originally suspected. The α1(I) collagen RNAs from cells and tissues actively engaged in synthesis of type I collagen display two major RNA species of about 5,000 and 5,200 bases and a minor species of about 7,100 bases (Fig. 3) (1, 17, 41). Thus there are at least three discrete RNAs in type I collagen-synthesizing cells which hybridize to the α1(I) collagen cDNA and which are apparently derived from the α1(I) collagen gene. Since the chicken α1(I) collagen gene has not yet been isolated, the relationships among these RNAs have not yet been determined. Analogy with the α2(I) collagen gene (4, 33) suggests that at least some of the transcript heterogeneity may be due to utilization of multiple polyadenylation signals. We cannot rule out, however, the possibility of multiple splicing pathways, similar to those observed for the rat and human fibronectin genes (22, 48).

The α1(I) collagen RNAs from normal chondrocytes, which are not normally engaged in type I collagen synthesis, display different sizes from those in other cells: a major RNA of 5,600 bases and a minor RNA of around 10,000 bases (Fig. 3) (16). A discrete 7,100-base RNA has never been observed in these cells. Most of our hybridizations have been performed under conditions of very high stringency (50% formamide–0.3 M NaCl at 50°C, washes in 0.015 M NaCl at 65°C). Thus we feel that these RNAs probably represent actual transcripts of the α1(I) collagen gene, rather than cross-hybridization of pCOL3 to other collagen RNAs. However, we cannot unequivocally rule out the possibility that the 5,600-base RNA represents cross-hybridization to type II collagen RNA, since it is similar to type II collagen RNA in size, and since pCOL3, the α1(I) collagen cDNA used as a probe in these experiments, encodes a carbohydrate attachment site that is highly conserved among types I, II, and III collagens (12, 35, 44, 50, 61). However, the minor 10,000-base RNA detected in chondrocytes has not been observed in any other cell type or with any other collagen probe (including those for the types II and III collagen genes) (16). Furthermore, no RNA of this size has been observed with a probe for type IX collagen, one of the minor cartilage collagens (34). Thus if the 10,000-base RNA represents cross-hybridization to another collagen RNA, it must be one of those for which probes are not yet available.

It seems likely to us that the size differences of the type I collagen RNAs in chondrocytes are due to alternative processing of the primary transcripts. If this is the case, then the restoration of the fibroblast-like RNAs in the transformed chondrocytes indicates that viral transformation not only alters the amounts of RNAs derived from the type I collagen genes, but also alters the way the primary transcripts are processed. The observation of a very large (probably 18,000 to 20,000 bases) α1(I) collagen RNA in transformed chondrocytes was somewhat surprising; such a large α1(I) collagen RNA has never been observed in any other cell type. Since the human α1(I) collagen gene is 18,000 base pairs in size (9), this very large RNA may represent a primary transcript of the α1(I) collagen gene. Its presence would imply that, although the processing of α1(I) collagen RNA in transformed chondrocytes is similar to that in fibroblasts, it may be less efficient.

Control of collagen gene expression. Type I collagen is a nearly ubiquitous protein, synthesized in most connective tissues (but not in most cartilages). The inability of cultured chondrocytes to synthesize type I collagen appears to be due to several factors. Normal chondrocytes (and those infected with ts-RSV and grown at the nonpermissive temperature for transformation) display low levels of type I collagen mRNAs (20% or less of that in skin fibroblasts); these RNAs have different electrophoretic mobilities from those found in fibroblasts and other cell types that actively synthesize type I collagen (Fig. 3 and 4) (16). These RNAs are not translated efficiently in vitro, since their products are not detected in proportion to the amount of RNA, and they are apparently not translated at all in the intact cells (16).

The aberrant expression of type I collagen genes in chondrocytes is partially abrogated by transformation with RSV, which results in increased amounts (Fig. 2) and altered electrophoretic mobilities (Fig. 3 and 4) of these RNAs. The type I collagen RNAs in transformed chondrocytes resemble, both in terms of amount and electrophoretic mobility, the RNAs found in fibroblasts (and other cells actively engaged in type I collagen synthesis). These RNAs are translated efficiently in vitro into identifiable type I collagen proteins, indicating that they meet the quantitative and structural requirements for translatability, which those from normal chondrocytes apparently do not. However, these RNAs are still not efficiently utilized in the intact cells, since no synthesis of the protein is detected with the culture conditions employed in these experiments.

There are several possible explanations for the inability of either normal or transformed chondrocytes to synthesize type I collagen. A particularly attractive hypothesis derives from the recent finding that the translation initiation regions of the types I and III collagens (62), but not type II collagen (21), mRNAs all have a highly conserved inverted repeat sequence with the potential for forming a stable stem-and-loop structure with the AUG start codon in the stem. One could postulate that a positive effector is required for translation of these RNAs to make this AUG codon available for translation initiation, and that chondrocytes, whether normal or transformed, are lacking such a factor. The lack of the inverted repeat sequence in the translation initiation region of type II collagen RNA (21) would free this RNA from the requirement for such a factor, thus permitting type II collagen synthesis in chondrocytes.

One could also postulate that chondrocytes possess a

negative effector that prevents translation of type I collagen RNAs, for example, by preventing ribosome movement through the stem-and-loop structure. This would seem more consistent with the translatability of these RNAs in heterologous systems such as the rabbit reticulocyte lysate; at the moment, however, there are no data that permit us to choose between these two mechanisms.

It should be noted that it is possible to override the translational control of type I collagen mRNAs in chondrocytes. Extended in vitro growth and treatment with bromodeoxyuridine, retinoic acid, tumor promoters, and fibronectin induce the synthesis of type I collagen protein in these cells (5, 27, 29, 40, 49, 55, 58) (our unpublished data). In addition, preliminary results from our laboratories and from others (18) indicate that transformed chondrocytes may initiate synthesis of type I collagen when they are grown as substrate-attached cells for extended periods of time. Thus these cells have apparently lost the ability possessed by both normal and transformed chondrocytes grown in suspension to discriminate against type I collagen mRNAs.

The data described here, in conjunction with those described elsewhere (16), indicate the existence of several levels at which synthesis of type I collagen may be regulated: (i) control of steady-state RNA levels, probably through a combination of transcription and degradation; (ii) control of RNA processing; (iii) control of translation by intrinsic structural features of the RNA; and (iv) control of translation by specific cytoplasmic factors that recognize the type I collagen mRNAs, either specifically enhancing or inhibiting their translation. This multiplicity of control levels may be important in developmental programs characterized by a switch from one collagen type to another. For example, during limb bud chondrogenesis, progenitor chondrogenic cells synthesizing type I collagen apparently differentiate into cartilage, synthesizing type II collagen and no type I collagen (11, 26, 54, 56). It is possible that the type I collagen genes, once activated, cannot be completely inactivated at the level of transcription; thus other regulatory mechanisms have evolved at posttranscriptional levels to prevent synthesis of type I collagen in tissues such as cartilage. This is consistent with the finding that the DNA methylation and nuclease hypersensitivity of the type I collagen genes do not differ substantially between tissues that synthesize and do not synthesize type I collagen (30, 32). The experimental system we have described will allow us to examine the mechanisms of the four control levels described above and to determine the relative contributions of each to the control of type I collagen gene expression during cell differentiation.

The complexity of regulation of the type I collagen genes is in striking contrast to the regulation of the type II collagen gene, which appears to be exerted exclusively at the level of transcription or RNA stabilization. Type II collagen is synthesized only in a limited number of terminally differentiated cell types. We have no evidence for transcription of the type II collagen RNAs in any cells other than those actively engaged in synthesis of type II collagen protein (16). In addition, the decreased type II collagen synthesis in transformed chondrocytes appears to be a direct reflection of the decreased type II collagen RNAs.

Based on the experimental observations described above, we suggest that the collagen genes may fall into two classes, in terms of control mechanisms. The genes encoding collagen proteins that are relatively ubiquitous, exemplified here by type I collagen, but probably also including type III (our unpublished observations), appear to be transcribed constitutively; thus they may require a multiplicity of posttranscriptional mechanisms to modulate the level of expression of the genes. Interestingly, the types I and III collagen genes have many structural features in common that may contribute to these control mechanisms. For example, all three of these genes possess the inverted repeat sequence within the region expected for ribosome binding and translation initiation referred to above (62); all three have several AUG codons upstream from the translation start site (62); and all three encode multiple RNA species (1, 16, 17, 41, 63), at least in part due to utilization of multiple polyadenylation signals (4, 33). The genes encoding collagen proteins that are synthesized solely as the products of a small number of terminally differentiated cell types (exemplified here by type II collagen, but perhaps including other cartilage-specific collagens) appear to be regulated predominantly by transcription or RNA stabilization or by both. The type II collagen gene does not possess an inverted repeat sequence in the translation initiation region (21) and does not possess multiple consensus signals for polyadenylation (35, 44). Furthermore, although there is one report of multiple type II collagen RNAs (57), recent reports from other laboratories have not shown this (16, 21, 28, 65).

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