Role of Procollagen mRNA Levels in Controlling the Rate of Procollagen Synthesis

LUCY B. ROWE AND RICHARD I. SCHWARZ*

The Jackson Laboratory, Bar Harbor, Maine 04609

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Two factors must be present for primary avian tendon cells to commit 50% of their total protein production to procollagen: ascorbate and high cell density. Scorbatic primary avian tendon cells at high cell density (>4 × 10⁶ cells per cm²) responded to the addition of ascorbate by a sixfold increase in the rate of procollagen synthesis. The kinetics were biphasic, showing a slow increase during the first 12 h followed by a more rapid rise to a maximum after 36 to 48 h. In contrast, after ascorbate addition, the level of accumulated cytoplasmic procollagen mRNA (α2) showed a 12-h lag followed by a slow linear increase requiring 60 to 72 h to reach full induction. At all stages of the induction process, the relative increase in the rate of procollagen synthesis over the uninduced state exceeded the relative increase in the accumulation of procollagen mRNA. A similar delay in mRNA induction was observed when the cells were grown in an ascorbate-containing medium but the cell density was allowed to increase. In all cases, the rate of procollagen synthesis peaked ~24 h before the maximum accumulation of procollagen mRNA. The kinetics for the increase in procollagen synthesis are not, therefore, in agreement with the simple model that mRNA levels are the rate-limiting factor in the collagen pathway. We propose that the primary control point is at a later step. Further support for this idea comes from inhibitor studies, using α,α'-dipyridyl to block ascorbate action. In the presence of 0.3 mM α,α'-dipyridyl there was a specific two- to threefold decrease in procollagen production after 4 h, but this was unaccompanied by a drop in procollagen mRNA levels. Therefore, inhibitor studies give further support to the idea that primary action of ascorbate is to release a post-translational block.

Primary avian tendon (PAT) cells at high cell density (>4 × 10⁶ cells per cm²) and in the presence of ascorbate (50 μg/ml) allocate half of their protein production to procollagen (22, 26). On the other hand, PAT cells at low cell density or in a medium deficient in ascorbate will produce only 12% procollagen (22, 26). This shift has previously been shown to be specific to procollagen production; the rate of noncollagen protein synthesis is identical in induced and uninduced cells (26). The molecular events, however, that regulate the commitment of PAT cells to the expression of a tissue-specific function have not been identified. Two basic hypotheses exist to explain how procollagen synthesis could be induced by ascorbate and high cell density. The first can broadly be described as transcriptional control (17). Essentially, this model requires that the controlling step regulating the rate of procollagen synthesis be the accumulated level of procollagen mRNA. Support for this model comes from studies in which chicken embryo fibroblasts were transformed with Rous sarcoma virus. After transformation, both procollagen mRNA levels and rates of procollagen synthesis declined, in what appeared to be a coordinate fashion (21, 27). A similar coordinate decline was observed when chick tendon cells were grown under cell culture conditions that inhibited full collagen expression (20, 22). An alternative hypothesis is that the collagen biosynthetic pathway is primarily controlled at a post-transcriptional point, with mRNA levels being altered by a feedback loop. This model does not specify a direct relationship between the level of specific mRNA and the rate of synthesis of the protein. Support for this type of control mechanism comes from the fact that the only established direct interaction of ascorbate with the collagen pathway is at a post-translational step—proline hydroxylation. Nevertheless, this is not sufficient evidence to rule out the alternative that ascorbate could act directly on the rate of transcription of procollagen mRNA through an unknown mechanism.

Distinguishing between a primary control mechanism acting at the transcriptional level and one acting at a post-translational step is the
aim of this study. Two approaches were used to bring evidence to bear that would discriminate between these models. The first was to examine the induction kinetics for a stimulation in the rate of procollagen synthesis after ascorbate addition or an increase in cell density, and compare this with the rate at which procollagen mRNA accumulates in the cytoplasm. The second approach was to inhibit ascorbate action in fully induced PAT cells and compare the decline in the rate of procollagen synthesis with the changes in the level of procollagen mRNA. The data are all consistent with the primary control step being post-translational.

MATERIALS AND METHODS

Cell culture. PAT cells were isolated from 16-day chicken embryos by a modification (22) of the Dehm and Prockop (8) procedure. PAT cells were grown in F12 medium (10) with 0.2% fetal calf serum in 25-cm² flasks as described previously (24, 26).

Collagen assay. PAT cells were labeled with [3H]proline (5'; Amersham Corp.) as described in the figure legends. Collagen production was determined by a collagenase assay (23) modified from that described by Peterkofsky and Diegelmann (18). Bacterial collagenase, used in this assay, recognizes a four-amino acid sequence that is, to a first approximation, only found in the collagen part of the procollagen molecule. This highly repetitive sequence in the collagen part of the procollagen molecule is degraded by collagenase and thereby detected (23). Our assay only measures collagen sequences, and we mathematically compensate for the fact that collagen is first made as a procollagen molecule (see legend to Fig. 1). The collagenase was purified to the extent that 50 to 100-fold excess enzyme could be used in the assay without evidence of general proteolytic activity (23).

Cytoplasmic RNA extraction. The procedures of Berger et al. (3) were modified for the small numbers of cells obtained from cell culture flasks. Briefly, the plasma membrane was lysed with Triton X-100 (0.5%) in a low-salt Tris buffer (20 mM Tris [pH 7.4], 10 mM NaCl, 3 mM magnesium acetate, 1.25% sucrose) containing the ribonuclease inhibitor, vanadyl ribonucleoside complexes. To concentrate the RNA, the lysate buffer (0.5 ml) was transferred from flask to flask (three flasks total, 3 × 10⁶ to 6 × 10⁶ cells). Under these conditions, the nuclei and cytoskeletons remained attached to the flasks. To the cytosol was added 43 μl of 10X ACE buffer (0.5 M sodium acetate; 0.1 M Na₂EDTA, pH 5.1) and 27 μl of sodium dodecyl sulfate (10%), and this solution was then phenol extracted. The yield was approximately 10 μg of RNA per flask.

Procollagen mRNA assay. The Thomas modification (28) of the Kafatos et al. (13) dot hybridization procedure was used to assay procollagen mRNA. In this procedure, the cytoplastic RNA (0.1 to 0.5 μg/2 μl) was boiled for 1 min and immediately spotted under a heat lamp onto the four corners of a high-salt-treated nitrocellulose filter (1.5 by 1.5 cm) and then baked in a vacuum oven (2, 80°C). After prehybridization for >4 h at 42°C, the RNA dots were hybridized in siliconized scintillation vials for 20 h at 42°C with gentle shaking and with 6 × 10⁶ cpm per filter of nick-translated [3²P]DNA of the plasmid pCg45 (pBRS22) with a 2.5-kilobase insert of procollagen α₂ cDNA sequences [16, 31] with a specific activity of 1 × 10⁶ to 4 × 10⁶ cpm/μg. The hybridized dots were washed, punched out, and counted in a scintillation counter. Two modifications of the Thomas procedure were made. First, the hybridization conditions were changed to be more stringent (70% formamide; 0.1 M PIPES [piperazine-N,N'bis(2-ethanesulfonic acid)], pH 6.4; 0.14 M NaCl; and 100 μg of sheared herring sperm DNA per ml) (21). This slightly improved the level of specific hybridization. More importantly, we found that it was necessary to quantify the amount of RNA retained on the filter at the end of the hybridization procedure since variations as great as two- to threefold in the level of RNA bound to the filter could be observed even with the same RNA sample. To reduce this source of error, the RNA was labeled by incubating PAT cells with 10 μCi of [3H]uridine (1 μl of medium) per flask for 2 h before extraction. The specific activity of the RNA was determined and the amount of RNA remaining bound at the end of the assay was calculated from the [3H] counts on the filter.

With these modifications, the assay is highly specific and is linear with both increasing total RNA spotted and with increasing concentrations of specific RNA in a spot. The limits of the assay for total RNA in a dot are defined by the background level on the low end and by the inability of more than 1 μg of RNA per spot to hybridize effectively. The limits of the assay for concentration of collagen-specific mRNA in a spot were explored by diluting a maximally induced RNA sample with RNA from yeast over a 20-fold range, and no loss of linearity was observed. Background intensities of hybridization were measured on dots of 0.5 μg of yeast or Escherichia coli RNA and were generally 6- to 15-fold below the lowest collagen mRNA containing signals measured in these experiments.

Northern blots. Total RNA (1 to 5 μg) was denatured with glyoxal and electrophoresed on 1% agarose gels as described by Thomas (28). The location of ribosomal RNA bands was determined by cutting a lane off one side of the gel and staining it with 0.2% methylene blue (in 0.2 M sodium acetate–0.2 M acetic acid) for 18 h and then destaining it with water (50°C). The remainder of the gel was blotted onto nitrocellulose paper as described by Thomas (28). Hybridization was as described above except that the prehybridization time was extended to 20 h, and the nick-translated probe was further purified by prebinding to glass fiber filters as described previously (4).

Whole cell RNA extraction. Total cellular RNA was extracted by lysing cells with 0.5% sodium dodecyl sulfate in freshly prepared 1× ACE buffer with vanadyl ribonucleoside complexes, rinsing 3 times with small volumes of this lysis mixture, and then extracting with phenol. Yields of RNA by this method were approximately 30 μg per flask.

α,α'-Dipyridyl experiments. Cells growing with and without ascorbate were given 0.3 mM α,α'-dipyridyl (14) in fresh medium at time zero. At various times thereafter, cells were pulsed for 2 h with [3H]proline (40 μCi per flask) to assay for procollagen synthesis or with [3H]uridine (10 μCi per flask) for cytoplasmic RNA extraction as described above.
RESULTS

Time-course for procollagen induction by ascorbate. PAT cells deprived of ascorbate devote approximately 12% of their total protein synthesis to procollagen (22, 26). When ascorbate is added to the medium, the level of procollagen synthesis rises over the course of 48 h to about 48% (22). To better understand the mechanisms behind this induction process, we analyzed in detail the kinetics for the increase in procollagen synthesis after ascorbate addition.

Experimentally, PAT cells were made scorbutic by growth in ascorbate-free medium for 4 days. On day 5, when nearly confluent (23), they were induced with ascorbate. At various times up to 60 h, cells were pulsed for 2 h with $[^3]H$proline and the percentage of newly synthesized procollagen was determined. A composite of the data from seven experiments is shown in Fig. 1A. Owing to the fact that ascorbate does not affect the rate of noncollagen protein synthesis (26) and owing to the fact that the incorporation of $[^3]H$proline into procollagen is linear (see Fig. 2), we can also plot the data as a function of the rate of procollagen synthesis (Fig. 1B). The induction by ascorbate was a slow process with biphasic kinetics. The initial effect of ascorbate on the cells was a slow increase in procollagen synthesis. After 12 h, the rate of the induction process accelerated so that a maximum rate of synthesis was reached by 36 to 48 h.

To assure that the pulse data accurately reflected synthesis patterns, we analyzed the ability of PAT cells to accumulate procollagen before and after ascorbate induction. Figure 2 shows the continuous incorporation of $[^3]H$proline into procollagen over a 24-h period. Both the fully induced and uninduced cells accumulated procollagen linearly; however, in this experiment, the rate was 8-fold greater (the mean value from several experiments being 6.8) in the induced cells. Scorbusic PAT cells given ascorbate at the start of the experiment showed an increasing rate of incorporation in good agreement with the pulse experiment (Fig. 1B). In Fig. 2 we have also included data obtained from fully induced PAT cells that were placed in ascorbate-free medium at the beginning of the experiment. The data show that PAT cells could continue to synthesize procollagen at a high rate without fresh ascorbate for an additional 12 h before slowing down. Combining this with the fact that ascorbate has a very short half-life in medium at 39°C (1.25 h) (6, 7) and that the cells last received ascorbate 24 h before the start of the experiment, one can conclude that the cells can accumulate enough ascorbate for maximum procollagen synthesis for approximately 36 h.

Time-course for procollagen mRNA (α2) induction. Having determined the time course for the increase in procollagen translation after ascorbate addition, one can then ask to what extent does procollagen mRNA concentration control this rate? To begin to answer this question, we performed a time-course study after ascorbate addition in which cytoplasmic RNA was extracted, bound to filters, and hybridized with a nick-translated procollagen $[^3]P$cDNA probe. In the experiment shown in Fig. 3, assays were made every 3 h up to 48 h after addition of ascorbate.
There was a 12-h delay before a linear rise. At 48 h the induction of procollagen mRNA was about two-thirds complete. As described below, full induction of procollagen mRNA required 60 to 72 h (see Fig. 5).

A similar time-course of RNAs was examined, using as a DNA probe plasmid pCg54, which is plasmid pBr322 with 1.1 kilobase of cDNA sequences from the procollagen α1 mRNA (15). The kinetics of increase in mRNA levels are essentially the same with the pCg45 (α2) probe (data not shown).

Northern blots. To confirm that the dot blot assay was working correctly, that we were indeed looking at cytoplasmic RNA, and that induction did not cause a change in the size of the procollagen mRNA, we analyzed RNA samples by agarose gel electrophoresis. We tested RNA from both induced and uninduced PAT cells that had either been extracted from the whole cell or only from the cytoplasm. We transferred the RNA from the gel to high-salt-treated nitrocellulose, hybridized with nick-translated probe, and autoradiographed the blot (Fig. 4). From whole cell extractions, two bands hybridized with our procollagen (α2) cDNA probe, in agreement with previous results reported by Adams et al. (1) and Wozney et al. (31). The induction of both bands (the relative proportions remaining constant; see legend to Fig. 4) is readily apparent and when quantitated by counting the bands in a scintillation counter gave equivalent results to that described for the dot blot assay (see legend to Fig. 4). The cytoplasmic RNA appeared as a single band at the lower molecular weight although degradation was apparent. These data further support the original interpretation by Adams et al. (1) that the higher-molecular-weight species was a precursor form. Again, with cytoplasmic RNA the induction was readily observed.

Relationship of procollagen mRNA levels to rate of procollagen synthesis. If procollagen mRNA was rate limiting in the collagen biosynthetic pathway, a direct relationship between the level of procollagen mRNA and the rate of procollagen synthesis would be predicted. Figure 5 shows the results obtained when both procollagen mRNA levels and rates of procollagen synthesis were analyzed together. Procollagen mRNA levels were determined at short time intervals (2 h) in the early part of the experiment and thereafter every 6 h up to 60 h to better assess the important changes taking place early and late in the induction process. To further aid comparison, we have normalized the data to the uninduced state and asked how the relationship established in scorbutic cells between mRNA

![Graph](chart.png)

**FIG. 2.** Accumulation of newly synthesized procollagen by PAT cells with and without ascorbate. PAT cells were incubated in 5 ml of medium containing 40 μCl of [3H]proline. At various times up to 26 h, the incubation was stopped and incorporation of radioactivity into procollagen (cells plus medium) was measured. The counts were corrected as described in the legend to Fig. 1. Four conditions were analyzed: fully induced cells (○); uninduced cells (□); uninduced cells plus ascorbate (▲); and fully induced cells minus ascorbate (▼).

**FIG. 3.** Accumulation of procollagen α2 mRNA after ascorbate addition. PAT cells were given ascorbate at time zero, and cytoplasmic RNA was extracted at various times and hybridized with nick-translated pCg45 cloned [32P]DNA, using a dot hybridization technique as described in the text. The level of specific hybridizable counts was analyzed from two independent determinations and plotted as the average. The mean induced level (−) (two determinations) and the fully induced level (+) (four determinations) are shown as dashed lines.
levels and rates of procollagen synthesis changed after ascorbate induction.

The ascorbate induction process could be divided into four phases. The first was the initial 12 h. During this period, the rate of procollagen synthesis rose slowly, whereas the level of procollagen mRNA showed a plateau. In the experiment illustrated in Fig. 5, there was actually a small decrease in apparent cytoplasmic procollagen mRNA. In other experiments, however, only a plateau was observed, and this was also true when whole cell RNA, rather than cytoplasmic RNA, was extracted (unpublished data).

The critical point is that no increase was observed in procollagen mRNA during the first 12 h. During the next period, from 12 to 48 h, both translation rates and mRNA accumulation increased. The relative rise in the rate of procollagen synthesis was much greater than in the first phase and exceeded the relative rise in procollagen mRNA accumulation. Moreover, at times during the induction process almost twice as much procollagen was being made per procollagen mRNA molecule than in uninduced cells. In the third period, 48 to 60 h, the rate of procollagen synthesis leveled off whereas the accumulation of procollagen mRNA continued to rise linearly. After time periods greater than 60 to 72 h the final phase was reached, in which both the rate of procollagen synthesis and level of procollagen mRNA were at maximum levels, approximately sixfold higher than in the uninduced control.

Induction by high cell density. Both ascorbate and high cell density are required for procollagen induction; ascorbate is clearly the easier
Generation time was by simply growing op fluency. ascorbate from was 0.8 since was lum slow relative was 1.6 \times 10^6 cells per flask. (A) Initial inoculum was 1.6 \times 10^6 cells per flask. (B) Initial inoculum was 0.8 \times 10^6 cells per flask.

variable to manipulate and has been analyzed first. However, since PAT cells grow with a 24-h generation time under standard conditions (24) and since the induction process by ascorbate was slow relative to this, a reasonable kinetic picture for induction by cell density could develop by simply growing the cells in the presence of ascorbate from low cell density up to confluency. As shown in Fig. 6B, the kinetics of cell density induction are strikingly similar to previous results (Fig. 5) where ascorbate was the variable in question. Again, full induction required an additional 24 h for procollagen mRNA to reach maximum levels when compared with the peak in the rate of procollagen synthesis. As was also seen in the ascorbate induction experiments, at each stage of the induction process mRNA levels trailed the rise observed in procollagen translation rates.

In a variation of the cell density experiment, we observed the effect of inoculating cells at a higher initial cell density. We knew from previous results (22) that this yields an extremely uniform cell culture where one could expect to observe maximum induction. In addition, we knew that PAT cells maintained at high cell density would not continue to express high levels of procollagen indefinitely (24). To explore the effect of maintaining cells at high cell density, we inoculated flasks at twice the normal level of cells, grew the cells in medium containing ascorbate, and followed the induction kinetics from day 3 to day 7. The cells were confluent on day 3 and showed a highly induced level (fivefold) for both procollagen mRNA and for procollagen translation rates (Fig. 6A). However, under these conditions the induction continued and reached a 10-fold level on day 5 before declining on days 6 and 7. Again, procollagen mRNA levels trailed by 24 h and peaked on day 6 and then declined. Between days 5 and 6, mRNA levels were increasing at the same time that the rate of synthesis of procollagen was declining.

Effects of inhibiting ascorbate action by \(\alpha,\alpha'\)-dipyridyl. The compound \(\alpha,\alpha'\)-dipyridyl is a ferrous ion chelator. Ferrous ion is required for the step in the collagen pathway where proline residues are hydroxylated. Consequently, on addition of \(\alpha,\alpha'\)-dipyridyl to tendon cell cultures, the proline hydroxylation reaction is inhibited. On the other hand, ascorbate is postulated to stimulate the hydroxylation reaction by ensuring that the iron within the cell is in the

\begin{figure}
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\includegraphics[width=\textwidth]{figure6}
\caption{Induction by high cell density of the rate of procollagen synthesis (\textbullet{}) and of the accumulation of procollagen mRNA (\textdagger{}). PAT cells were grown in F12 medium plus 0.2% serum and ascorbate (50 \(\mu\)g/ml) and the medium was changed daily. The induction was normalized to cells treated identically except that no ascorbate was added to the medium. (A) Initial inoculum was 1.6 \times 10^6 cells per flask. (B) Initial inoculum was 0.8 \times 10^6 cells per flask.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7}
\caption{Effect of adding \(\alpha,\alpha'\)-dipyridyl (0.3 mM) to fully induced (\textbullet{}) and uninduced (\textdagger{}) PAT cells. The rates of procollagen synthesis have been normalized to that of noncollagen protein synthesis to correct for nonspecific inhibition of protein synthesis (30 to 50% in 6 h). Each point in the graph reflects a 2-h pulse ending at the time indicated. The controls (without \(\alpha,\alpha'\)-dipyridyl) are shown as dashed lines \(\pm\) SD.}
\end{figure}
reduced ferrous state (2, 32). Addition of α,α'-dipyridyl to PAT cells should therefore be a simple mechanism for rapidly reversing this action of ascorbate. Previous research by Kao et al. (14) on the effects of α,α'-dipyridyl on procollagen secretion rates led them to conclude from indirect evidence that this inhibitor also suppresses procollagen translation rates. If this is indeed true, one could then ask whether this inhibition of procollagen translation was mediated through procollagen mRNA levels.

To answer this question, we first examined whether α,α'-dipyridyl would rapidly inhibit the rate of procollagen synthesis in fully induced cells. As a control, we also examined the effects of α,α'-dipyridyl on uninduced (scorbutic) PAT cells. This compound non-specifically inhibited protein synthesis in scorbutic cells by 30 to 50% after 6 h; the percentage of total protein synthesis devoted to procollagen remained constant. In fully induced cells, however, the effect of chelating ferrous ion was highly specific for procollagen synthesis, causing an additional two- to threefold inhibition in 4 h (Fig. 7). No further reduction was observed up to 8 h, the longest time tested (data not shown). Thus, α,α'-dipyridyl rapidly inhibited the high rate of procollagen synthesis induced by ascorbate.

To determine whether this drop in procollagen translation rates was mediated through a decline in mRNA levels, we measured the levels of procollagen mRNA in the cytoplasm in both fully induced and uninduced cells after treatment for 6 h with α,α'-dipyridyl. The presence of α,α'-dipyridyl did not reduce the procollagen mRNA levels (Table 1).

**DISCUSSION**

The role played by procollagen mRNA levels in controlling the rate of procollagen synthesis cannot be reduced to a simple proportional relationship. Although the full 6- to 10-fold increase in procollagen production by either ascorbate addition or increasing cell density probably requires a similar 6- to 8-fold increase in procollagen mRNA levels, the kinetic data are not in agreement with the simple model which predicts that mRNA levels are controlling synthetic rates. In the induction process, the increase in mRNA levels starts 12 h later and reaches a maximum 12 to 24 h after the increase observed in the rate of procollagen translation. This can lead to an anomalous situation in which procollagen mRNA levels can be increasing while procollagen translation rates are actually decreasing (Fig. 6A). One is led to conclude that the level of mRNA may be important for full induction but it is not the primary controlling step in the collagen biosynthetic pathway.

This lack of a one-to-one correspondence be-

<table>
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<th>Cells</th>
<th>3P (cpm) bound per μg of RNA</th>
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<tbody>
<tr>
<td>−C</td>
<td>5,448 ± 1,121</td>
</tr>
<tr>
<td>−C + α,α'-dipyridyl</td>
<td>4,818 ± 1,021</td>
</tr>
<tr>
<td>+C</td>
<td>34,089 ± 3,848</td>
</tr>
<tr>
<td>+C + α,α'-dipyridyl</td>
<td>37,346 ± 12,589</td>
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* Cytoplasmic RNA was isolated from induced and uninduced cells that had been treated for 6 h with α,α'-dipyridyl (0.3 mM) and compared with untreated controls. The RNA was dot hybridized with nick-translated pCG45 probe. The hybridized dots were cut out and counted on a scintillation counter and listed in the table as the mean ± SD. −C, Uninduced cells; +C, cells fully induced with ascorbate.

between procollagen mRNA levels and the rate of procollagen synthesis is not a unique observation. Tolstoshev et al. (29) noted that in human lung fibroblasts the level of procollagen mRNA varied twofold as the cells grew from low cell density to confluent cultures; yet the rate of procollagen production remained constant. Tolstoshev et al. (30), in another communication, noted that in sheep skin the percentage of procollagen synthesis dropped from 14% in early development to 2% at term; however, during the same period, procollagen mRNA levels remained constant. These observations, although in less well-defined systems, support our conclusion that there need not be a direct correspondence between procollagen mRNA levels and the rate of procollagen synthesis, particularly when mRNA levels appear to be in excess.

This idea is further supported by our studies with the inhibitor α,α'-dipyridyl. In this case, a rapid inhibition of ascorbate action caused a two- to threefold decline in the rate of procollagen synthesis, but the level of procollagen mRNA remained constant. In other words, one can again separate the influence of mRNA levels from being the principle regulatory agent controlling the rate of translation.

The control in the α,α'-dipyridyl experiment, the scorbutic cell, is critical. In this case, procollagen synthesis is not specifically inhibited by α,α'-dipyridyl. This is strong evidence that the inhibitor has high specificity for reversing the action of ascorbate and not just translation in general. In addition, this specificity of α,α'-dipyridyl for the induced cell confirms the general assumption that the interaction of ascorbate with the collagen pathway is related to its ability to insure a supply of ferrous iron.

Further elucidation of the primary control step in the collagen pathway requires an understanding of the events that occur during the first 12 h after ascorbate addition. Although only small changes occur during this period in the
accumulation and translation of procollagen mRNA, ascorbate causes important changes in the collagen pathway (19) at other steps. Ascorbate rapidly enters the cell and acts as a cofactor for the increased hydroxylation of proline residues in the completed procollagen molecule (7, 14, 26). The exact time-course of this increase in hydroxylation has not been precisely determined; however, indirect evidence would have ascorbate causing full hydroxylation in less than 1 h (14, 26). As a result of an increase in proline hydroxylation, the triple helical conformation of the collagen portion of the procollagen molecule is stabilized. The triple helical procollagen molecule is more rapidly secreted from the cell (11, 12). An increase in secretion rates with only a nominal increase in the rate of procollagen translation causes a net drop in the internal procollagen pool size. We have previously shown that increased secretion, and not the rate of proline hydroxylation, correlates with the induction of procollagen synthesis (26). Thus, secretion rates could be playing a primary role in regulating the pathway. A major question is whether, and by what mechanism, these early changes after ascorbate addition feed back to bring about the increased rate of procollagen synthesis and the accumulation of procollagen mRNA.

Further support for this type of model, in which the primary control step is post-translational, comes from previous experiments that showed that increasing cell density also stimulates increased rates of procollagen secretion (26). There appears, therefore, to be a common link between factors that can act as inducers in this system. Moreover, we have shown that the induction kinetics by either ascorbate or high cell density are very similar for procollagen synthesis or mRNA accumulation. The implication is that they are affecting similar or closely linked steps in the pathway. Whether the critical step is indeed the rate of procollagen secretion remains to be more firmly established.

What is clear is that a tendon cell needs to precisely regulate collagen production because collagen production is ultimately related to the length of the tendon. Tendon cells overproducing collagen would yield a tendon that was too long, and underproducers, a tendon too short. Only the correct level of collagen synthesis will yield a functional tissue (5). The ability of PAT cells to react to both ascorbate and cell density is probably a reflection of the need of the organism to precisely control collagen production. Understanding how PAT cells regulate this tissue-specific function should aid in explaining how other proteins are developmentally controlled.

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LITERATURE CITED


