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Communication

Declining Procollagen mRNA Sequences in Chick Embryo Fibroblasts Infected with Rous Sarcoma Virus

CORRELATION WITH PROCOLLAGEN SYNTHESIS*

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SUMMARY

Chick cells infected with Rous sarcoma virus are characterized by a wide variety of changes known collectively as transformation. Among these are decreases in the level of procollagen biosynthesis and in the level of procollagen mRNA. In this communication, we examine the time course of the decrease in procollagen biosynthesis, as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and collag enase assay, and compare it with the decrease in procollagen mRNA sequences measured by hybridization to a complementary DNA. Procollagen biosynthesis and procollagen mRNA sequences decrease simultaneously after infection. Even the initial decrease in procollagen biosynthesis, therefore, is due to a decline in the level of procollagen mRNA.

The study of cellular changes induced by viral infection may elucidate cellular mechanisms in viral replication and in the normal regulation of host cell macromolecular synthesis. In eukaryotes, viral infection may or may not be followed by production of virus, and if production does ensue, it may be passive or lytic in nature. Thus, the extent to which host function is disrupted is dependent on the nature of the infection. For example, herpes simplex virus causes degeneration of poly(A)-containing RNA within 4 h in lytically infected Friend leukemia cells (1). In the case of poliovirus, host mRNA, although retained, is no longer translated because of changes in the host's translation machinery (2). On the other hand, Rous sarcoma virus induces a variety of changes in chick embryo cells, collectively known as transformation (3), and utilizes a relatively small percentage of host cell synthetic machinery for its replication (4).

A number of specific host functions, as well as the distribution of various proteins and mRNA sequences, are altered following transformation by RSV

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The abbreviations used are: RSV, Rous sarcoma virus; SDS, sodium dodecyl sulfate; ts, temperature-sensitive.

shown that both synthesis of the protein and the procollagen mRNA level, when monitored by in vitro translation or hybridization to procollagen cDNA, are reduced in transformed cells (10, 17, 18).

In this study, we have measured the kinetics of the decrease in procollagen biosynthesis and in procollagen mRNA sequences during the first 84 h after infection of chick embryo fibroblasts with RSV. Specifically, we were interested in whether this initial reduction in procollagen synthesis could be attributed to events which affect the concentration of the message or to factors altering the activity of this mRNA. Our results indicate that procollagen synthesis is decreasing by 24 h after infection and that the time course of the decline in procollagen mRNA sequences and procollagen synthesis is similar.

EXPERIMENTAL PROCEDURES

Purification of Procollagen mRNA—Procollagen mRNA was purified from chick embryo tendons essentially as described by Rowe et al. (17). Total RNA was obtained by Proteinase K digestion in SDS followed by phenol/chloroform extraction. Total RNA was enriched for poly(A)-containing RNA by chromatography over oligo(dT)-cellulose. This RNA was further enriched for procollagen mRNA by size fractionation on sucrose gradients as described (17). Yields and enrichment for procollagen mRNA were similar to those obtained previously.

Translation of Procollagen mRNA—Translation was performed in a staphylococcal nuclease-treated rabbit reticulocyte lysate which had been chromatographed over Sephadex G-50. Conditions of translation were those optimized for procollagen synthesis by Rowe et al. (17). Products of in vitro translation were assayed by electrophoresis with procollagen standards in SDS-polyacrylamide gel electrophoresis as described by Laemmli (19), and observed by fluorography (20). Sensitivity to bacterial collagenase (Form III, Advance Biofactures) was determined by digestion as described by Peterkofs y and Diegelmann (21). The collagenase was shown to be free of nonspecific proteolytic activity.

Synthesis of [3H]cDNA—The mRNA template was selected from sucrose gradient fractions containing procollagen mRNA activities. cDNA was synthesized as described by Rowe et al. (17) and further purified by back-hybridization to polyosomal RNA, enriched for procollagen mRNA sequences by immunoprecipitation with procollagen antibodies as described by Lee et al. (22). Fifty micrograms of immunoprecipitated polyosomal RNA was hybridized to 100,000 cpm of procollagen cDNA for 14 h at 68°C using reaction conditions described by McKnight and Schimke (23). The hybridized cDNA was then chromatographed on hydroxyapatite followed by Sephadex G-75 chromatography to remove single-stranded cDNA and salt, respectively. About 40% of the counts per min in cDNA was recovered after back-hybridization to immunoprecipitated polyosomal RNA. Double-stranded cdNA-RNA hybrids were base-treated in 0.3 M NaOH overnight to remove RNA. This purification increased saturation from about 80% to almost 68%.

Hybridization of cDNA—Hybridization reactions and quantitation of hybrids using S1 nuclease were carried out essentially as described (29).

Fibroblast Culture Conditions—Tendon fibroblasts were explanted from 17-day chick cells not susceptible to infection by sarcoma virus subgroup E (H and N Farms, Redmond, Wash.) according to the method of Delm and Procop (24). Cells were maintained in F-12 medium (GRIMCO), 5% calf serum, and 10% trypsin/P, broth in an atmosphere of 5% CO2 at 30°C.

Kinetics of Infection—Cells were trypsinized and plated at a density of 1 x 106 cells/60 mm plastic tissue culture dish (Falcon). To one set of cells, an additional milliliter of medium was added, containing 1 x 104 focus forming units of Prague C strain of RSV and 10 µg of polybrene (both obtained as gifts from Robert Eisenman, Fred.

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Hutchinson Cancer Research Center, Seattle, Wash.). Control dishes received an additional milliliter of medium supplemented with polybrene alone. Medium was changed at 24 and 48 h. Cells were observed every 12 h after infection. At the times indicated, one infected and one control plate were washed three times with Dulbecco’s modified Eagle’s medium, preincubated in Dulbecco’s modified Eagle’s medium, supplemented with 50 μg/ml of ascorbate for 1 h, and then pulsed for 30 min with 2 ml of the latter medium supplemented with 25 μCi/ml of [2,3-^3H]proline. At the end of the pulse, each cell layer was divided into equal parts; half was dissolved in 0.5 M ammonium hydroxide and processed for protein determinations (25), collagenase digestion (21), and SDS-polyacrylamide gel electrophoresis (19), and half was taken up in SET buffer (1% SDS, 5 mM EDTA, 10 mM Tris, pH 7.5) containing 50 μg/ml of Proteinase K in SDS and extracted in phenol/chloroform as described above.

Cell layers dissolved in 0.5 M ammonium hydroxide were precipitated with trichloroacetic acid for collagenase assay as described by Peterkofsky and Diegelmann (21). The supernatants of this precipitation were counted to determine the radioactivity in proline pools in normal and transformed cells. Trichloroacetic acid-soluble pools of proline, expressed per μg of protein, in transformed cells were 1.4 times those in normal cells by 84 h. This could reflect larger pools of proline or higher specific activity of proline pools in transformed cells, or both. If the specific activity of the proline pools is not affected by transformation, then changes in collagenase-sensitive counts per min in proline, per μg of protein, would reflect absolute differences in collagen synthesized in normal and transformed cells. If, on the other hand, the specific activity of proline pools is greater in transformed cells, then measurement of collagenase-sensitive proline, expressed per μg of protein, may actually inflate the apparent level of collagen made by transformed cells, relative to normal cells. Data expressed as the percentage of total counts per min which is collagenase-sensitive are, of course, unaffected by differences between the specific activity of proline pools in normal and transformed cells.

**RESULTS**

**Purification of Procollagen mRNA and Synthesis of Procollagen cDNA**—Translation of sucrose gradient-fractionated chick tendon poly(A)-containing RNA revealed a broad region of procollagen mRNA activity spanning the marker 28 S rRNA peak (data not shown). Electrophoresis of translation products on SDS-polyacrylamide gels (Fig. 1, Slot I) showed that the mRNA activity in this region of the gradient stimulated the synthesis of proteins migrating in the region of chick proc(1) and pro(2) markers. The existence of doublets in each of these positions was variable and could have been due to partial proteolysis of the procollagens by the lysate itself. Fig. 1 demonstrates that these bands were sensitive to bacterial collagenase; the products obtained by translation of oviduct mRNA were not (data not shown). Interestingly, some mRNA template activity for pro(2) migrated perceptibly earlier in the sucrose gradient than did proc(1) activity (data not shown). Procollagen cDNA was synthesized from pooled fractions of the gradient containing mRNA activity for both pro(2) and proc(1) chains.

**Kinetics of the Decrease in Procollagen Synthesis Following Infection with RSV**—The time course of the decline in procollagen synthesis in chick embryo tendon fibroblasts following infection with RSV is shown in Fig. 2. Procollagen synthesis, as indicated by collagenase-sensitive proline-labeled protein in the cell layer, declined starting 24 h after infection...
that in the control cells (Fig. 3, Slot I). By 84 h, transformed gen bands of the transformed cells (Fig. 3, Slot 5) was 43% of that, at 48 h after infection, radioactivity in the two procollagen biosynthesis can be accounted for by a corresponding decrease in procollagen mRNA levels. The decrease in procollagen mRNA levels shown here could result from decreased synthesis, increased degradation, or a combination of these factors. Experiments are underway to distinguish among these possibilities.

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