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Regulation of Procollagen Messenger Ribonucleic Acid Levels in Rous Sarcoma Virus Transformed Chick Embryo Fibroblasts†

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ABSTRACT: Using cloned cDNAs for pro-α1 and pro-α2 collagen messenger ribonucleic acid (mRNA), we have investigated the regulation of collagen mRNA levels in Rous sarcoma virus (RSV) transformed chick embryo fibroblasts (CEF). We find that both pro-α1 and pro-α2 mRNA levels are decreased ~10-fold in CEF transformed by either the Bryan high-titer strain or the Schmidt–Ruppin strain of RSV. Using temperature-sensitive mutants in the transforming gene *src*, we also investigated the rate of change in the levels of the two mRNA species. We employed mutants of both the Bryan high-titer strain (BHTa) and the Schmidt–Ruppin strain (ts68). With both mutants the results were similar. Upon shift from the permissive temperature (35 °C) to the nonpermissive temperature (41 °C), collagen mRNA synthesis did not increase until more than 5 h had passed, suggesting the action of *src* on collagen gene expression is indirect. Upon shift from 41 to 35 °C, collagen mRNA levels fell with a half-life of 10 h. Whether this fall reflects the half-life of procollagen mRNA or an effect of *src* on procollagen RNA stability is unclear. Both pro-α1 and pro-α2 mRNA levels were coordinately controlled.

Fibroblasts which are transformed by ribonucleic acid (RNA)[1] tumor viruses show a variety of altered properties (Hanafusa, 1977; Pastan & Willingham, 1978). One major alteration is the decrease in collagen gene expression which follows transformation of chick embryo fibroblasts (CEF)[1] by Rous sarcoma virus (RSV). We have been investigating this transformation parameter in order to understand at a molecular level some of the events that result in the abnormal properties of neoplastic cells.

Several laboratories have reported a decrease in procollagen synthesis in various transformed cell lines (Green et al., 1966; Hata & Peterkofsky, 1977; Levinson et al., 1975; Kamine & Rubin, 1977; Peterkofsky & Prather, 1974; Schwartz et al.,

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[1] Abbreviations used: RNA, ribonucleic acid; mRNA, messenger RNA; RSV, Rous sarcoma virus; CEF, chick embryo fibroblasts; RSV–CEF, RSV-transformed CEF; ts, temperature sensitive; SR, Schmidt-Ruppin strain of RSV; BH, Bryan high-titer strain of RSV; DBM, diaminobenzyloxymethyl; SSC, 0.15 M sodium chloride and 0.015 M sodium citrate; $NaDodSO_4$, sodium dodecyl sulfate; cDNA, complementary deoxyribonucleic acid; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; poly(A), poly(adenylic acid).

1978). Studies with RSV-transformed CEF (RSV–CEF) have demonstrated a correlation between decreased type I procollagen synthesis and a reduction in translatable procollagen mRNA (Adams et al., 1977). Recently, more direct evidence has been presented to indicate that collagen mRNA levels are decreased in RSV–CEF. Howard et al. (1978) and Sobel et al. (1978) used RNA extracted from CEF and RSV–CEF to synthesize specific collagen cDNA restriction fragments. They found that restriction fragments characteristic of collagen cDNA are synthesized in reduced amount when RSV–CEF RNA is the template. Rowe et al. (1978) demonstrated decreased levels of procollagen mRNA in RSV–CEF by using partially purified chick procollagen cDNA as a probe in liquid hybridization studies, and Sandmeyer & Bornstein (1979) showed that procollagen biosynthesis and procollagen mRNA sequences decrease simultaneously after infection of CEF by RSV.

Type I procollagen consists of three polypeptides, one pro-α2 collagen chain and two identical pro-α1 chains. We have reported the construction and characterization of two recombinant bacterial plasmids, one of which contains a chick pro-α2 collagen cDNA sequence (Sobel et al., 1978) and the other of which contains a chick pro-α1 collagen cDNA sequence (Yamamoto et al., 1980). In each case, the cloned cDNA sequences were positively identified by specific hybridization to either pro-α2 collagen mRNA (Sobel et al., 1978; Adams et al., 1979) or pro-α1 collagen mRNA (Yamamoto et al., 1980; Adams et al., 1979). With these cloned sequences as hybridization probes, Adams et al. (1979) showed that pro-α1 and pro-α2 collagen RNA species are reduced in RSV–CEF.

In the experiments reported here, we have investigated the time course of the change in steady-state mRNA levels in RSV–CEF by using two strains of RSV which contain temperature-sensitive (ts) mutations in the transforming gene *src*. Cells infected with these mutant viruses have a normal phenotype when propagated at the nonpermissive temperature of 41 °C but undergo rapid morphologic and metabolic changes when placed at a permissive temperature of 34–36 °C (Pastan & Willingham, 1978). One virus, ts68, is a mutant of the Schmidt–Ruppin (SR) strain of RSV (Kawai & Hanafusa, 1971); the other, BHTa, is a mutant of the Bryan high-titer (BH) strain of RSV (Bader, 1972). In both cases, the mutation does not affect the growth of the virus. Using the cloned pro-α1 and pro-α2 collagen DNA sequences as hybridization probes to quantify type I procollagen RNA levels, we find that procollagen mRNA decreases relatively rapidly within the first 10 h after the shift from the nonpermissive to the permissive temperature for transformation. On the other hand, when ts68- or BHTa-infected CEF are shifted from a transformation-permissive temperature to 41 °C, procollagen mRNA levels do not begin to increase significantly for 10 h.[2]

Experimental Procedures

Cells and Viruses. CEF were propagated and routinely grown at 39 °C in GM medium (Vogt, 1969) and were transformed with either the wild-type SR or BH strains of RSV. CEF infected with ts68 (Kawai & Hanafusa, 1971) or BHTa (Bader, 1972), the respective variants of SR and BH which are ts for transformation, were initially grown at 37 °C. Cells infected with the wild-type RSV strains exhibited a transformed morphologic phenotype (Pastan & Willingham, 1978) at all temperatures between 35 and 41 °C. CEF infected with either ts68 or BHTa exhibited a transformed phenotype at 35 °C and a normal morphology at 41 °C. Temperature shifts were made during the second and/or third passage and are described in detail in the text. Third passage cells were planted at 1×10^7 cells/150-mm (Falcon) dish and grown for 2 days before harvesting. In each experiment, all cells were derived from the same embryo. CEF infected with ts68 and BHTa grew at the same rate regardless of the incubation temperature.

Preparation and Electrophoresis of RNA. Total cellular RNA was extracted from third passage CEF by an adaptation of the guanidine procedure (Strohman et al., 1977) described previously (Adams et al., 1977). Concentration of RNA was measured by absorption at 260 nm in a Gilford spectrophotometer, Model 240. Total cellular RNAs were electrophoresed in a 3 mm thick horizontal slab gel of 1% agarose (Sigma Chemical Co., Type II) containing 6 mM methylmercuric hydroxide (Alfa) for 2 h at 50 V measured across the gel as described (Adams et al., 1979; Bailey & Davidson, 1976).

Transfer of RNA to Diazobenzyloxymethyl (DBM) Paper. Aminobenzyloxymethyl paper was prepared in the laboratory of J. Alwine (NIH) from Whatman 540 paper as described (Alwine et al., 1977) by using 1-[[(*m*-nitrobenzyl)oxy]-methyl]pyridinium chloride which was a gift from J. Alwine. Diazotization of aminobenzyloxymethyl paper to DBM paper was as described (Alwine et al., 1977). After electrophoresis of RNA on methylmercury–agarose gels, the RNAs were transferred covalently to DBM paper (Alwine et al., 1977). Complete transfer of RNA from the gel to the DBM paper was tested by staining the gel after transfer with ethidium bromide. Quantitative transfer of RNA to similarly prepared DBM paper has been previously demonstrated (Adams et al., 1979; Alwine et al., 1977).

Recombinant Bacterial Plasmids. Recombinant plasmid pCOL1 contains a 200 base pair pro-α2 collagen cDNA sequence (Sobel et al., 1978). Plasmid pCOL3 contains an 800 base pair pro-α1 collagen cDNA sequence (Yamamoto et al., 1980). The recombinant plasmids were propagated in *Escherichia coli* χ1776 as described (Yamamoto et al., 1980) in accordance with the National Institutes of Health guidelines for recombinant DNA research.

Preparation of Procollagen DNA Hybridization Probes. Supercoiled pCOL1 and pCOL3 plasmid DNAs were purified from *E. coli* χ1776 after equilibrium centrifugation in cesium chloride–ethidium bromide (Yamamoto et al., 1980). For purification of the pro-α2 collagen DNA insert of pCOL1, the plasmid DNA was first restricted with the endonucleases *Bam*HI and *Eco*RI (Sobel et al., 1978). The restricted DNA was then electrophoresed on a 7% polyacrylamide gel and eluted (Sobel et al., 1978). For purification of the pro-α1 collagen DNA insert of pCOL3, the plasmid DNA was restricted with the endonuclease *Hind*III, electrophoresed, and eluted. Purified pro-α1 and pro-α2 collagen DNA was nick translated with [γ-^{32}P]ATP as described (Maniatis et al., 1975).

Hybridization of RNAs to Procollagen DNA. After transfer of total cellular RNAs to DBM paper, the DBM filters were pretreated for 3 h at 37 °C in a sealed polyethylene bag containing hybridization buffer and 0.5 mg/mL yeast RNA (Adams et al., 1979). ^{32}P-Labeled procollagen DNA was denatured by boiling for 5 min and added to the filter without changing the hybridization buffer. Hybridization was for 18–36 h at 37 °C (Adams et al., 1979). Filters were washed 6 times for 15 min each at 37 °C with 1 × SSC

[2] A preliminary report of this work was presented at the annual meeting of the Federation of American Societies for Experimental Biology.

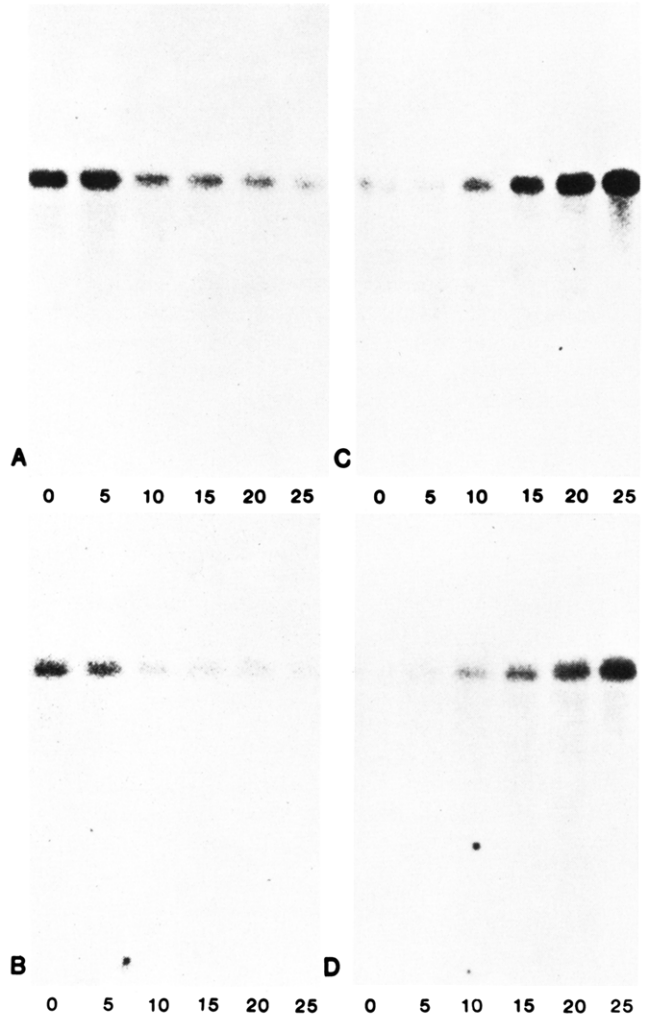

FIGURE 1: Autoradiographs of DBM filters identifying procollagen RNAs in ts68-infected CEF grown at different temperatures. Cells infected with ts68 were initially grown at 37 °C before temperature shift, as described under Experimental Procedures. (A and B) CEF infected with ts68 were grown at 41 °C, and then parallel cultures were shifted to 35 °C for different intervals before harvesting. (C and D) CEF infected with ts68 were grown at 35 °C, and then parallel cultures were shifted to 41 °C for different intervals before harvesting. Total cellular RNA was extracted from each set of cultures at the same time, and 5 μg of each RNA preparation was electrophoresed on 1% agarose horizontal slab gels containing 6 mM methylmercury as described under Experimental Procedures. To ensure that equal amounts of RNA were loaded in each lane, we stained the gel with ethidium bromide and assessed the quantities of 18S and 27S ribosomal RNAs. The RNAs were transferred to DBM paper, hybridized to ^{32}P-labeled cloned pro-α1 collagen DNA (A and C) or to ^{32}P-labeled cloned pro-α2 collagen DNA (B and D), and autoradiographed as described under Experimental Procedures. The number of hours after temperature shift are below each lane.

containing 2 mM EDTA and 0.2% $NaDodSO_4$. After autoradiography, hybridized DNA was removed from the DBM filters by treating with 99% formamide at 70 °C for 5 min. The DBM filters with the covalently attached RNA species could then be rehybridized to a different ^{32}P-labeled procollagen DNA probe in fresh hybridization buffer. Thus each RNA–DBM-cellulose preparation was reused without affecting the hybridization kinetics.

Autoradiography. Washed DBM filters after hybridization were exposed for varying periods of time to Kodak RP/2 Royal X-Omat film at −70 °C. Autoradiographs were scanned with a Joyce-Loebl microdensitometer, and areas under the peaks were determined with an electronic planimeter (Numonics Corp.).

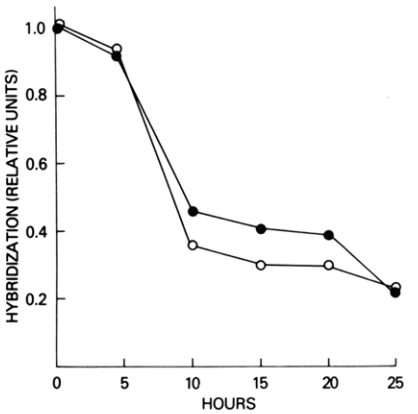

FIGURE 2: Relative quantities of pro-α1 and pro-α2 collagen RNAs in ts68-infected CEF grown for different periods of time at 35 °C. The autoradiographs from Figure 1A,B were scanned with a microdensitometer, and the areas under the peaks were determined with an electronic planimeter as described under Experimental Procedures. Autoradiographs were exposed for varying lengths of time to ensure that the exposure times used were within the linear response range for all RNA species. Similar data were derived from autoradiographs developed from at least two other DBM filters and from DBM filters containing one complete set of independently isolated RNA preparations. Relative amounts of hybridized pro-α1 collagen RNAs (●) and pro-α2 collagen RNAs (○) are plotted against the number of hours of growth after the shift of ts68-infected CEF from 41 to 35 °C. A value of 1.0 was assumed for the amount of hybridized procollagen RNA present before the shift to 35 °C.

Results

Decrease in Procollagen RNA Levels in ts68-Infected CEF after Shift to Transformation-Permissive Temperature. CEF infected with ts68 were grown at 41 °C for 72 h to achieve steady-state levels of procollagen mRNA. When viewed by phase contrast microscopy, the cells looked like normal CEF. Cells were shifted from 41 to 35 °C at different times, and total cellular RNA was extracted at 5-h intervals after temperature shift. A total of 5 μg of each RNA preparation was fractionated on a 1% agarose slab gel containing 6 mM methylmercury and covalently transferred to DBM paper. Figure 1A,B presents autoradiographs of the DBM filter after it was hybridized to ^{32}P-labeled pro-α1 collagen DNA (Figure 1A) and pro-α2 collagen DNA (Figure 1B). In cells maintained at 41 °C throughout the experiment (0 h), the major procollagen RNA species detected by this method are identical in size with those observed in normal CEF and in poly(A)-containing calvaria RNA enriched for procollagen mRNA (Adams et al., 1979). The pro-α1 collagen DNA probe hybridizes a major RNA species with a length of ~5000 bases. In addition, in some experiments and with prolonged exposure, a minor RNA species with an approximate size of 7100 bases is visualized (e.g., Figure 4). The pro-α2 collagen DNA probe hybridizes to two major RNA species of 5200 and 5700 bases, which are not separated well in the autoradiographs presented. As shown in Figure 1A,B, a marked decrease in pro-α1 and pro-α2 collagen RNA levels was evident at 10 h after a shift down to the transformation temperature.

To quantify this effect, we scanned each of the lanes in Figure 1 with a densitometer and measured the areas under the curves. In Figure 2, the relative quantities of hybridizable pro-α1 and pro-α2 collagen RNAs are plotted against a time course of hours of growth at the transformation-permissive temperature. The pattern clearly shows that there is a small fall in procollagen mRNA levels at 5 h and a much greater decrease in both pro-α1 and pro-α2 collagen RNA levels at 10 h. However, after 10 h a new steady-state level of procollagen RNAs appears to be maintained. This new steady-

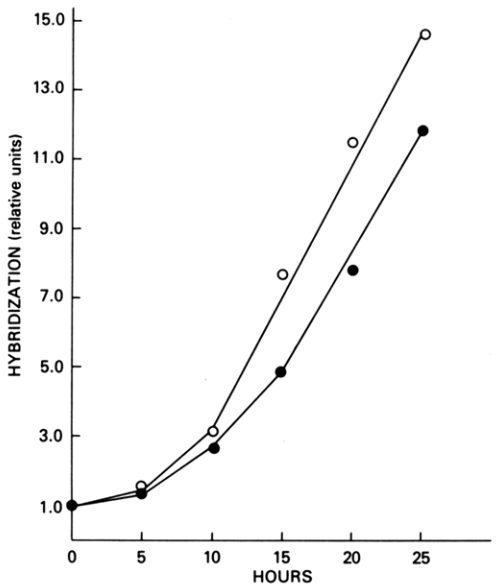

FIGURE 3: Relative quantities of pro-α1 and pro-α2 collagen RNAs in ts68-infected CEF grown for different periods of time at 41 °C. The autoradiographs in Figure 1C,D were scanned with a microdensitometer, and the areas under the peaks were determined with a planimeter as described in Figure 2. Relative amounts of hybridized pro-α1 collagen RNAs (●) and pro-α2 collagen RNAs (○) are plotted against the number of hours of growth after the shift of ts68-infected cells from 35 to 41 °C. A value of 1.0 was assumed for the amount of hybridized procollagen RNA present before the shift to 41 °C.

state level is ~40% of that present before temperature shift. A much larger difference in levels of collagen mRNA is observed when normal and wild-type SR-transformed CEF are compared [see Table I and Adams et al. (1979)], indicating that the transformation phenotype is somewhat leaky in ts68-infected CEF under these conditions.

Increase in Procollagen RNA Levels in ts68-Infected CEF after Shift to Transformation-Nonpermissive Temperature. In similarly designed experiments, the time course of alteration from transformed to normal phenotype was followed by switching the temperature of ts68-infected cells from the transformation-permissive temperature to the restrictive temperature. Third passage CEF infected with ts68 were grown at 35 °C for at least 48 h, during which time they exhibited the morphologic phenotype of transformation (Pastan & Willingham, 1978). The temperature of parallel cultures was shifted up from 35 to 41 °C at different times, and the RNA was analyzed at 5-h intervals. Panels C and D of Figure 1 present autoradiographs of DBM filters after hybridization to ^{32}P-labeled pro-α1 and pro-α2 collagen DNA probes, respectively. Figure 3 shows the relative quantities of procollagen RNAs plotted against a time course of hours of growth at the temperature restrictive for transformation. Procollagen RNA levels are only slightly increased after 5 h and then increase at a linear rate after 10 h. However, a new steady-state level of procollagen RNAs is not achieved even 20 h after the temperature shift.

Effect of Temperature on Procollagen RNA Levels in Normal and SR-Transformed CEF. To demonstrate that the variations in procollagen RNA levels observed in ts68-infected cells were not due to a direct effect of temperature on collagen gene expression, we switched parallel cultures of normal CEF and CEF transformed with the wild-type SR strain of RSV which had been growing at 39 °C to either a 35 or a 41 °C incubator and grew the cultures for an additional 20 h. Total cellular RNA was extracted, fractionated, and analyzed as described above. Figure 4 is an autoradiograph of the DBM

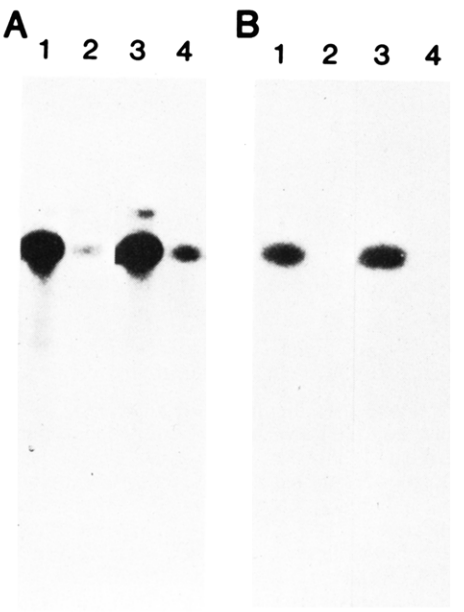

FIGURE 4: Autoradiograph of DBM filters identifying procollagen RNAs in CEF and SR-transformed CEF grown at different temperatures. Total cellular RNAs from CEF grown for 20 h at 35 °C (lane 1) or 41 °C (lane 3) and from SR-transformed CEF grown for 20 h at 35 °C (lane 2) or 41 °C (lane 4) were fractionated and transferred to DBM paper as described in Figure 1. (Panel A) Autoradiograph of RNAs hybridized to ^{32}P-labeled cloned pro-α1 collagen DNA. (Panel B) Autoradiograph of RNAs hybridized to ^{32}P-labeled cloned pro-α2 collagen DNA.

Table I: Effect of Temperature on Relative Quantities of Procollagen RNAs in Normal and SR-Transformed CEF[a]

cells	growth temp (°C)	hybridized pro-α1 collagen RNA	hybridized pro-α2 collagen RNA
CEF	35	1.00	1.00
SR-CEF	35	0.16	0.12
CEF	41	1.07	1.14
SR-CEF	41	0.24	0.17

[a] The autoradiographs from Figure 4 were scanned with a microdensitometer, and the areas under the peaks were determined with an electronic planimeter as described under Experimental Procedures. Autoradiographs were exposed for varying lengths of time to ensure that the exposure times used for these calculations were within the linear response range for all RNA species. A value of 1.00 was assumed for the amount of hybridized procollagen RNA present in CEF grown at 35 °C.

filter after hybridization to ^{32}P-labeled pro-α1 collagen DNA (Figure 4A) or to pro-α2 collagen DNA (Figure 4B). The procollagen RNA species in uninfected CEF grown at either 35 °C (lane 1) or 41 °C (lane 3) are identical with those observed in ts68-infected cells. These species are barely detectable in SR-transformed CEF grown at either 35 °C (lane 2) or 41 °C (lane 4). The quantities of hybridizable pro-α1 and pro-α2 collagen RNA species relative to the amount of procollagen RNA present in uninfected CEF grown at 35 °C are summarized in Table I. Only a minor increase in procollagen RNA levels occurs when cells are grown at 41 °C. This effect is true of both normal and SR-transformed CEF. Therefore, we conclude that the effect of temperature shift on procollagen RNA levels in ts68-infected CEF is due directly or indirectly to the action of the *src* gene product.

Changes in Procollagen RNA Levels in BHTa-Infected CEF. CEF transformed with the Bryan high-titer strain of RSV exhibit a slightly different morphologic phenotype from

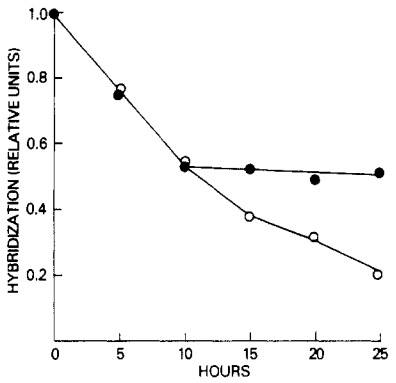

FIGURE 5: Relative quantities of pro-α1 and pro-α2 collagen RNAs in BHTa-infected CEF grown for different periods of time at 35 °C. CEF infected with BHTa were grown at 41 °C for at least 72 h, and parallel cultures were shifted to 35 °C for different intervals before harvesting. Total cellular RNA was fractionated, transfered to DBM paper, and hybridized to ^{32}P-labeled pro-α1 and pro-α2 collagen DNA probes as described in Figure 1. Relative amounts of hybridized pro-α1 collagen RNA (●) and pro-α2 collagen RNA (○) are plotted against the number of hours of growth after the shift from 41 to 35 °C as described in Figure 2. A value of 1.0 was assumed for the amount of hybridized procollagen RNA present before the shift to 35 °C.

that of SR-transformed CEF (Pastan & Willingham, 1978). When CEF infected with mutant BHTa are placed at the permissive temperature, the cells change shape and accumulate intracellular vacuoles within 10 min (Pastan & Willingham, 1978). This is a little more rapid than the morphologic change noted when ts68-infected cells are shifted to the permissive temperature (Pastan & Willingham, 1978). We therefore measured the decrease and increase in procollagen mRNA levels in BHTa-infected CEF grown at different temperatures. The relative quantities of hybridizable pro-α1 and pro-α2 collagen RNAs in the shift-down experiments are plotted as a function of time after temperature shift in Figure 5. There is a rapid decrease in procollagen RNAs within 5 h after the BHTa-infected CEF are shifted to the permissive temperature. The decrease for pro-α1 collagen RNA is linear for 10 h, and, then, as for ts68-infected CEF, the rate of decrease is markedly diminished. The decrease in pro-α2 collagen RNA species, however, continues to decrease over the time course of the experiment. Figure 6 is a plot of the relative quantities of pro-α1 and pro-α2 collagen RNAs in BHTa-infected CEF at varying intervals after a shift from the transformation-permissive to the restrictive temperature. This pattern is almost identical with that seen for ts68-infected cells in the shift-up experiment. The lack of correlation between pro-α1 and pro-α2 collagen RNAs at the later time points is most likely a reflection of the very low absolute amount of hybridization to pro-α2 collagen RNA at the 0 time point in these experiments, which is consistent with the continuous decrease in pro-α2 collagen levels depicted in Figure 5. The data in Table II indicate that the level of procollagen RNAs in wild-type BH-transformed CEF is not significantly affected by temperature shifts.

Discussion

In the experiments reported here, we measured the levels of pro-α1 and pro-α2 collagen mRNA in CEF transformed by two ts transformation mutants of RSV after shifts to and from the transformation-permissive and -nonpermissive temperatures. The levels of procollagen RNAs were measured by the sensitive gel-transfer DBM-filter hybridization technique of Alwine et al. (1977). As hybridization probes, we used specific cloned pro-α1 and pro-α2 collagen cDNA sequences.

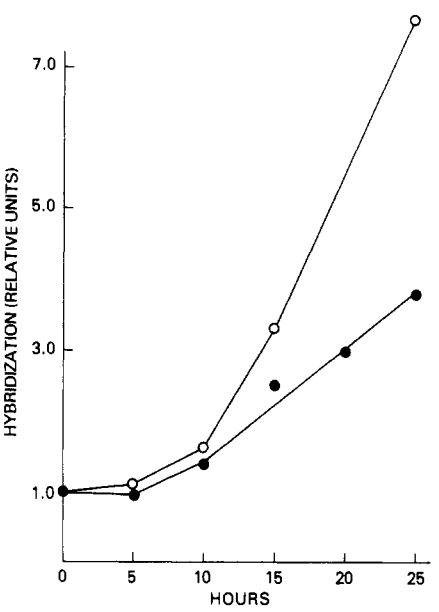

FIGURE 6: Relative quantities of pro-α1 and pro-α2 collagen RNAs in BHTa-infected CEF grown for different periods of time at 41 °C. CEF infected with BHTa were grown at 35 °C for at least 48 h, and parallel cultures were shifted to 41 °C for different intervals before harvesting. Total cellular RNA was fractionated, transferred to DBM paper, and hybridized to ^{32}P-labeled pro-α1 and pro-α2 collagen DNA as described in Figure 1. Relative amounts of hybridized pro-α1 collagen RNA (●) and pro-α2 collagen RNA (○) are plotted against the number of hours of growth after the shift from 35 to 41 °C as described in Figure 3. A value of 1.0 was assumed for the amount of hybridized procollagen RNA present before the shift to 41 °C.

Table II: Effect of Temperature on Relative Quantities of Procollagen RNAs in Normal and BH-Transformed CEF[a]

cells	growth temp (°C)	hybridized pro-α1 collagen RNA	hybridized pro-α2 collagen RNA
CEF	35	0.95	1.10
	39	1.00	1.00
	41	1.30	1.14
BH-CEF	35	0.16	0.06
	39	0.11	0.04
	41	0.19	0.09

[a] CEF and BH-transformed CEF were initially grown at 39 °C. Parallel cultures were shifted to either 35 or 41 °C for 25 h before total cellular RNA was extracted, fractionated, transferred to DBM paper, and hybridized to ^{32}P-labeled pro-α1 and pro-α2 collagen DNA. After autoradiography, the amount of hybridized procollagen RNA was determined by densitometry and planimetry as described in Table I. A value of 1.00 was assumed for the amount of hybridized procollagen RNA present in CEF grown at 39 °C.

When ts68- or BHTa-infected CEF are shifted to a transformation-permissive temperature, levels of both pro-α1 and pro-α2 collagen RNAs are decreased ~60% within 10 h. In ts68-infected cells, the decrease appears to occur after a short lag. There is no such lag in BHTa-infected cells, which may reflect a more rapid inactivation of the *src* gene product in this mutant. A new steady state of procollagen RNA levels is achieved 10 h after temperature shift. The difference in steady-state levels between normal and transformed phenotypes in these cells is less substantial than that between uninfected CEF and CEF transformed by the wild-type SR or BH strains of RSV. In some experiments, collagen mRNA levels in ts68-infected CEF grown at 41 °C for over 24 h were close to 100% of the levels found in normal CEF. Thus ts68-infected CEF grown at 35 °C have ~40% of the levels of collagen

mRNA found in normal CEF. This is 4 times the levels found in cells infected with wild-type RSV. This is most likely due to the "leaky" phenotype of the ts mutation. The decrease in procollagen RNA levels is slower than the changes in hexose uptake and morphology that have been observed (Pastan & Willingham, 1978; Kawai & Hanafusa, 1971; Bader, 1972) and is similar to the time course reported for the disappearance of fibronectin on the cell surface of CEF (Hynes & Wyke, 1975) and for the decrease of fibronectin mRNA levels (Fagan et al., 1980). In vitro translation experiments (data not shown) indicate that most other mRNA species are not decreased in amount after temperature shift to the permissive temperature, including such large mRNA species as myosin.

In contrast to the relatively rapid effect seen in the shift-down experiments, there is a long lag before procollagen RNA levels increase when ts68- or BHTa-infected CEF are shifted up to the transformation-nonpermissive temperature. A linear increase in procollagen RNA levels is not evident until at least 10 h after temperature shift. In vitro translation studies confirm these results (data not shown). Alterations in pro-α1 and pro-α2 collagen RNA levels followed the same initial pattern in the temperature-shift experiments reported here, indicating that these two distinct gene products are regulated by the same process. The further decrease in pro-α2 collagen mRNA levels at later time points in both SR- and BH-infected CEF may reflect a higher susceptibility to degradation of the pro-α2 collagen RNA molecule.

Transformation of cells by RSV may be considered as a model for the study of differentiation. Kaji and Holtzer and their co-workers have shown that RSV has specific effects on transcription when introduced into myogenic cells, melanoblasts, and chondrocytes (Holtzer et al., 1975; Boettiger et al., 1977; Pacifici et al., 1977; Muto et al., 1977). Investigations in our laboratory have centered on the effect of *src* on the regulation of fibronectin and collagen genes in CEF (Adams et al., 1977; Howard et al., 1978; Sobel et al., 1978; Adams et al., 1979; Fagan et al., 1979). The results reported here indicate that pretranslational control of procollagen RNAs plays a major role in procollagen gene regulation. The temperature-shift data suggest that the *src* gene product $p60^{src}$ either directly or indirectly acts on the differentiation program of CEF with a resultant decrease in procollagen mRNA levels. This decrease could be due to an effect of *src* on the synthesis, maturation, or degradation of procollagen mRNA.

The temperature-shift data do not provide direct information concerning the rates of procollagen mRNA synthesis and/or degradation. Reliable data on the half-life of procollagen mRNA are not yet available. If we speculate, however, that specific degradation plays no role in the decrease in procollagen mRNA levels seen after temperature shift to the permissive temperature and that transcription of the collagen genes ceases immediately after temperature shift, then shift-down data in Figures 2 and 3 indicate that the half-life of the procollagen mRNAs is <10 h. This calculation takes into account an average generation time of 20 h for normal and RSV-transformed CEF.

The data from the shift-up experiments, in which the increase in procollagen RNA levels is barely detectable until 10 h after temperature shift, indicate that if transcription plays a role in this phenomenon, it is a complex process. Possibly, the change from a transcriptionally inactive to an active procollagen gene may involve a change in conformation of the chromatin structure which is cell cycle dependent. Cell cycle dependent regulation of dihydrofolate reductase mRNA metabolism has recently been reported (Hendrickson et al., 1980). It is also possible, however, that an immediate increase in transcription rate is masked after temperature shift due to the simultaneous action of a persistent specific procollagen ribonuclease. Current experiments are directed toward testing some of these possibilities.

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Sequence Complexity of Nuclear and Cytoplasmic Ribonucleic Acids from Clonal Neurotumor Cell Lines and Brain Sections of the Rat†

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ABSTRACT: The sequence complexity of both nuclear and cytoplasmic ribonucleic acids (RNAs) from rat brain is higher than the sequence complexity of these RNAs from other tissues. In addition, a higher percentage of the nuclear RNA sequences appear as cytoplasmic RNA in brain than in other organs [Chikaraishi, D. M., Deeb, S. S., & Sueoka, N. (1978) *Cell (Cambridge, Mass.) 13*, 111–121; Chikaraishi, D. M. (1979) *Biochemistry 18*, 3249–3256]. We would like to determine whether the high sequence complexity of brain RNAs is found in every cell of the brain or if this high complexity is due to the summation of lower, incompletely overlapping, complexities in the various cell types of the brain. We measured the sequence complexity of RNA fractions from both neurotumor cell lines and brain sections by saturation hybridization. Nuclear RNA from the central nervous system cell lines B103 and B27 hybridized to 14.5 and 13.5% of the unique-sequence DNA, respectively, while nuclear RNA from the peripheral neurotumor cell line RT4-D1 hybridized to 11.1% of the unique-sequence DNA. If asymmetric transcription is assumed, these hybridization values correspond to complexities of 29.0, 27.0, and 22.2% of the unique-sequence genome or 5.3×10^8, 4.9×10^8, and 4.0×10^8 nucleotides for the three cell lines. The sequences transcribed into nuclear RNA in these cell lines are included in the set of sequences transcribed in whole brain. Nuclear RNA from the five brain sections examined hybridized to from 14.7 to 16.3% of the unique-sequence DNA, within experimental error of the 15.6% found for whole brain. Total cytoplasmic RNA from B103 and B27 cell lines hybridized to 3.1 and 4.3% of the unique-sequence DNA, respectively, while released polysomal RNA hybridized to 3.2 and 3.9% for the two cell lines. These cytoplasmic complexities are equivalent in each case to ~25% of the sequences appearing in the nucleus of the cell line. In contrast, total cytoplasmic and released polysomal RNAs from whole brain were previously shown to represent 50–65% of the sequences appearing in the nucleus. Some brain sections show complexities for polyadenylated cytoplasmic RNA lower than the value for whole brain. In particular, this RNA fraction hybridized to 2.8% of the unique-sequence DNA for cerebellum as compared to 5.1% for whole brain. These data support the view that most of the sequences transcribed into nuclear RNA of the brain are found in every cell of the brain but that only one-fourth to one-third of these sequences are eventually translated in any given cell. Therefore, there must be a great deal of posttranscriptional selection in the brain.

The sequence complexity of RNA[1] from adult tissues of several rodents has been measured by saturation hybridization. In the rat (Shearer, 1977; Chikaraishi et al., 1978; Grouse et al., 1978; Kaplan et al., 1978; Tedeschi et al., 1978; Chikaraishi, 1979; Grady et al., 1979; Lee et al., 1979; Wilkes et al., 1979), mouse (Hahn & Laird, 1971; Brown & Church, 1972; Grouse et al., 1972; Bantle & Hahn, 1976; Grady et al., 1978; Kuroiwa & Natori, 1979; Van Ness et al., 1979), and rabbit (Brown & Church, 1972; Church & Schultz, 1974) the brain has consistently shown a higher complexity in both its nuclear and cytoplasmic RNAs compared to that found in other tissues. This relatively high sequence complexity for brain could either result from higher sequence complexity in every brain cell or from the summation of lower complexities with different transcripts in the various cell types of the brain.

Data from our laboratory have shown that, for the rat, brain nuclear RNA hybridizes to 15.5%, liver nuclear RNA hybridizes to 11%, and kidney, spleen, and thymus nuclear RNAs each hybridize to ~5% of the unique-sequence DNA. If asymmetric transcription is assumed, these nuclear RNAs represent 31, 22, and 10% of the unique-sequence genome, respectively. If the high complexity observed in the brain was due to a summation of different transcripts in each of many cell types, one might expect the average number of copies of a given sequence per cell to be lower for the brain than for other organs. This is not, however, observed. Brain, liver, and

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[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; hnRNA, heterogeneous nuclear RNA; rRNA, ribosomal RNA; mRNA, messenger RNA; HAP, hydroxylapatite; [^{125}I]DNA or unique-sequence [^{125}I]DNA, ^{125}I-iodinated unique-sequence DNA; PBS, phosphate-buffered saline; poly(A)+, polyadenylated; R_0t, RNA concentration (moles of nucleotide per liter) at t_0 × time (seconds); $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Cl_3CCOOH, trichloroacetic acid.

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