Restriction of the Rat-specific RNA Sequences of Ki-MSV to the Nucleus of Dibutyryl Cyclic AMP-treated K-A31 Cells

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SUMMARY

The treatment of oncogenically transformed cells in culture, with dibutyryl cyclic AMP (cAMP) has, in many cases, resulted in a general phenotypic change towards the normal state. A virus-specific gene product(s) is responsible for the transformation of cells by sarcoma viruses and it has been suggested that the src gene product may act through the alteration of cAMP levels. With these premises we have studied the effects of dibutyryl cAMP on cell growth and virus genome expression in a Kirsten sarcoma virus-transformed mouse cell line. Our results suggest that certain virus-specific RNA sequences are restricted to the nucleus of these cells after several days of growth in medium containing dibutyryl cAMP and that these sequences appear to be those coding for the sarcoma information.

INTRODUCTION

The involvement of adenosine 3′:5′-cyclic monophosphate (cAMP) in the growth control of cultured cells, in particular those of the mouse and rat, is now well established (Pastan & Johnson, 1974; Pastan & Willingham, 1978). Oncogenically transformed cells have lower cAMP levels than their normal counterparts (Sheppard, 1972) and, in contrast to normal cells, those levels fail to increase at confluency (Otten et al. 1971; Carchman et al. 1974; Pastan & Johnson, 1974).

Treatment of transformed cells with compounds which elevate cellular cAMP levels, such as the analogue dibutyryl cyclic AMP (dbcAMP), can cause varying degrees of phenotypic changes towards the normal state and almost always results in a reduced growth rate (Johnson & Pastan, 1972; Pastan & Johnson, 1974). Furthermore, onset of the transformed character can be prevented in cells infected with a sarcoma virus, temperature-sensitive for transformation, if dbcAMP is present before switching from the non-permissive to the permissive temperature (Otten et al. 1972; Carchman et al. 1974). These observations have been interpreted as suggesting that the src gene product may act through the alteration of cAMP levels (Pastan et al. 1974). [Work done mostly with avian sarcoma viruses has shown that the product(s) of a specific virus gene, the src gene, is responsible for the onset and maintenance of the transformed phenotype; for review, see Vogt, 1977.]
Kirsten murine sarcoma virus (Ki-MSV) arose after passage of Kirsten murine leukaemia virus (Ki-MuLV) through rats (Kirsten & Mayer, 1967) where it acquired the ability to transform fibroblasts by recombination with rat cellular sequences (Scolnick et al. 1973). It is replication-defective and requires a leukaemia virus as helper. Here we present data which suggest that RNA transcripts specific to the rat sequences of Ki-MSV become restricted to the nucleus of Ki-MSV-transformed Balb/c mouse cells (K-A31) treated with dbcAMP.

METHODS

Cell culture. Cells were grown in 16 oz glass prescription bottles (surface area 70 cm²), in Dulbecco's modification of Eagle's minimal essential medium, supplemented with 10% foetal calf serum (complete medium). Cell lines used included Ki-MSV-transformed non-producer Balb/c embryonic cells (K-A31), NIH/3T3 cells producing Ki-MuLV and NIH/3T3 cells transformed by and producing Ki-MSV (Ki-MuLV). AA-ascites rat tumour cells (of spontaneous origin, obtained from Mason Research Institute, Mass., U.S.A.) were serially propagated at 8 day intervals in the peritoneal cavity of non-inbred Wistar rats.

DbcAMP treatment and assays of cell growth. K-A31 cells (2.5 x 10⁸) in complete medium were seeded into glass prescription bottles. Twenty-four h later and daily thereafter, cells were fed with complete medium containing 0.1 mM N6,O2'-dibutyryl adenosine 3':5'-cyclic monophosphoric acid and 1 mM theophylline (both from Sigma, St. Louis, Mo., U.S.A.), an inhibitor of cAMP phosphodiesterase. Cells were harvested for RNA extraction after 72 h growth in treated medium.

At suitable times and following procedures described above, replicate cultures were assayed for growth by cell counting or rate of DNA synthesis. For cell counting, cells were trypsinized, scraped with a rubber policeman when necessary and counted in a Coulter counter or by haemocytometer. To determine rates of DNA synthesis, cells were pulse-labelled for 30 min with 5 ml complete medium containing 1 μCi/ml [3H]thymidine (20 Ci/mmol; New England Nuclear, Boston, Mass., U.S.A.). Cells were rinsed, trypsinized, pelleted by centrifugation and resuspended in ice-cold phosphate-buffered saline (PBS). After aliquots were taken for cell counting (by haemocytometer), duplicate samples were made to 0.1% SDS, shaken well and adjusted to 10% trichloroacetic acid (TCA). The precipitate was pelleted, dissolved in 0.3 m-NaOH and incubated for 60 min at 37°C. Aliquots were again adjusted to 10% TCA to precipitate DNA, which was collected on filters, washed, dried and counted in omnifluor (New England Nuclear).

Reverse transcriptase assay. Spent culture media from control and dbcAMP-treated cells were processed and analysed for the presence of reverse transcriptase in an in vitro reaction using poly(rA).oligo(dT) as template-primer (Ayukawa et al. 1979).

RNA synthesis. Rates of heterogeneous nuclear RNA (hnRNA) synthesis were measured using the method of Brandhorst & McConkey (1974) with modifications (B. P. Brandhorst, personal communication). Briefly, cells were pulse-labelled for 10 min with 2-3H-adenosine (21.5 Ci/mmol; Amersham, Oakville, Ont., Canada) at a concentration of 25 μCi/ml in Hanks' basal salt solution; hnRNA is preferentially labelled by the short pulse (Houssais & Attardi, 1966; Soeiro et al. 1968). 2-3H-adenosine loses its tritium atom when converted to guanosine and does not exchange its tritium atom with water during experimental manipulations. Cells were rinsed and harvested by scraping with a rubber policeman while culture bottles were resting on ice. Cells were pelleted and homogenized in cold 10% TCA. The precipitate was collected, washed by centrifugation through perchloric acid and the RNA-specific activity (d/min/A₂₅₀) determined for alkali-labile material. Acid-extracted ATP was bound to and eluted from charcoal, purified by thin-layer chromatography, quantified by the firefly luciferin–luciferase assay and its radioactivity determined by scintillation counting.
Tritiated toluene standards were used to determine radioactivity in terms of disintegrations/min. Rates of synthesis were estimated by dividing the specific activity of the RNA by the average specific activity of the ATP pool during the pulse.

*RNA extraction.* Gram quantities of cells were collected after 72 h in complete medium containing 0.1 mM dbcAMP and 1 mM theophylline, by scraping in cold PBS with a rubber policeman, and stored at −70 °C until used.

Cytoplasmic RNA was obtained from cells shaken in lysis buffer and extracted repeatedly in phenol–chloroform, essentially as described (Fan & Baltimore, 1973). In addition, following alcohol precipitation and resuspension in buffer [0.02 M-Tris (pH 7.4); 0.05 M-NaCl; 0.001 M-magnesium acetate] samples were digested with DNase (20 μg/ml, RNase-free, 30 min, 21 °C), further extracted by phenol–chloroform and exhaustively dialysed. Nuclear pellets were stored at −70 °C until used. The RNA was lyophilized and resuspended in distilled water at a concentration of 10 to 20 mg/ml. Twenty-three 4000 units were taken to represent 1 mg/ml of RNA and all RNA used had A260/A280 ratios greater than 2. Yield was customarily 1 to 3 mg/g of cells.

Nuclear RNA and total cellular RNA were extracted essentially as described by Mukherjee & Mobry (1979). Briefly, samples were homogenized, phenol extracted, digested with DNase (as above) and Pronase (500 μg/ml, 90 min, 37 °C) and run through a CsCl gradient.

*Synthesis of Ki-MSV (Ki-MuLV) 3H-cDNA probe and nucleic acid hybridization.* Ki-MSV (Ki-MuLV) grown in NIH/3T3 cells and purified by zonal ultracentrifugation was a gift from Dr R. Benveniste, N.I.H., Bethesda. Virus-specific 3H-cDNA (a radiolabelled DNA probe complementary to the virus RNA genome) was synthesized from this virus in an endogenous reaction as described by Benveniste et al. (1977). Briefly, a reaction mixture containing 0.4 M-Tris (pH 7.8), 0.06 M-KCl, 12 mM-magnesium acetate, 2 × 10⁻³ M-dithiothreitol, 0.02% (v/v) Triton X-100, 50 μg/ml actinomycin D, 5 × 10⁻³ M each of dATP, dCTP and dGTP, and the purified virus (approx. 0.2 to 0.4 mg virus protein/ml) was incubated for 4 h at 37 °C. The product was deproteinized, alkali-digested for 12 h, neutralized and applied to a Sephadex G-50 column. Fractions containing acid-precipitable radioactivity were pooled, dialysed and lyophilized. The 3H-cDNA product prepared by this method had a specific activity of 1.8 × 10⁶ ct/min/μg (based on equimolar incorporation of dNTPs) and was routinely found to be 98% single-stranded. Hybridization of 3²P-labelled virus RNA to 3H-cDNA showed that the 3H-cDNA was a good representative and relatively uniform copy of the virus genome.

DNA–RNA hybridization reactions were performed essentially as described (Mukherjee & Mobry, 1979) and contained 0.01 M-Tris (pH 7.4), 0.6 M-NaCl, 10⁻⁵ M-EDTA, 0.05% sodium dodecyl sarcosinate, 60 μg/ml calf thymus DNA, approx. 5 × 10⁴ ct/min/ml of 3H-cDNA and 2 to 8 mg/ml of RNA. Hybridization mixtures in glass tubes under mineral oil were first heated to 100 °C for 10 min and then incubated at 65 °C. At varying times 0.02 ml amounts were withdrawn and kept at −70 °C until digested with single-strand-specific S₁ nuclease (Miles Laboratory, 1000 units per sample) in a buffer containing 0.033 M-sodium acetate (pH 4.5), 2 × 10⁻⁴ M-ZnSO₄, 0.13 M-NaCl and 30 μg/ml denatured calf thymus DNA. Hybridization was measured as the percentage of input 3H-cDNA counts that remained TCA-precipitable after S₁ nuclease digestion. R₀₉ (product of RNA concentration and reaction time) values were calculated as suggested by Britten et al. (1974) and corrected to a monovalent cation concentration of 0.18 M. A comparison of the relative concentration of virus-specific RNA in various cells was made by comparing the kinetics of hybridization using R₀₉ as described by Leong et al. (1972).

The 3H-cDNA–RNA hybrids formed under the hybridization conditions described were consistently found to be as well matched as the hybrids formed between 3H-cDNA and homologous virus RNA (based on melting temperature).
RESULTS

Cell growth and DNA synthesis in dbcAMP-treated and untreated K-A31 cells

The growth characteristics of K-A31 cells were analysed under the conditions used for growing and harvesting gram quantities. The combination of 0.1 mM-dbcAMP and 1 mM-theophylline was used for its effect on cell growth and lack of toxicity as indicated by the exclusion of trypan blue stain and the normal replating efficiency of the cells. Treated cells showed a decreased growth rate compared to controls and, although they did not become truly contact-inhibited, they reached a saturation density much lower than that of untreated cultures, which eventually reached a maximum and began sloughing off the growth surface (Fig. 1). Certain phenotypic changes characteristic of dbcAMP treatment (Pastan & Johnson, 1974; Pastan & Willingham, 1978) were also observed. Cells became more compacted and flattened-out, and their adhesion to the substratum was greatly increased. These changes occurred gradually and, like the change in growth rate, were more noticeable after 24 h of treatment.

Butyrate, which is a degradation product of dbcAMP, has been shown in certain cell lines to induce changes similar to those induced by dbcAMP although in some cases its action has been shown to be mediated by cellular levels of cAMP (Prasad & Sinha, 1976). To investigate the possibility that the causative agent in our treatments might be butyrate rather than dbcAMP, an additional group of cultures was treated with 0.2 mM-sodium butyrate (Baker, Phillipsburg, N.J., U.S.A.), the maximum concentration attainable if a 0.1 mM-dbcAMP solution was completely degraded. The results in Fig. 1 show clearly that the butyrate-treated cultures were similar to the untreated controls in their growth behaviour. The butyrate treatment had no observable effect on morphology or adhesion of cells to the substratum.

Fig. 1. Influence of dbcAMP, theophylline and sodium butyrate on the growth of K-A31 cells. Cells were inoculated into small plastic culture bottles (area 25 cm²) and fed with appropriate media 24 h later and daily thereafter. At indicated times cells were trypsinized and counted in a Coulter counter. Each point represents the average number of cells from four to seven bottles and is shown with its standard deviation. ●, Cells fed with control media; ■, cells fed with media containing 0.2 mM-sodium butyrate; ▲, cells fed with media containing 0.1 mM-dbcAMP and 1 mM-theophylline.
Nuclear restriction of Ki-MSV-specific RNA

Fig. 2. Influence of dbcAMP and theophylline on the incorporation of $^3$H-thymidine by K-A31 cells. □, Control; ‡, treated.

As another parameter of cell growth we examined the rate of incorporation of $^3$H-thymidine into DNA. As expected, DNA synthesis fell off markedly in treated cells (Fig. 2).

Reverse transcriptase assay of K-A31 cell culture fluid

To ensure that the K-A31 cells were not producing virus and that dbcAMP treatment was not inducing the production of virus, which would preclude an analysis of differential virus genome expression, assays were performed for reverse transcriptase on spent culture media. Whereas reactions containing purified viruses or processed media from producer cell cultures incorporated $^3$H-TTP into acid-precipitable material with high efficiency, all reactions containing samples of processed control and treated K-A31 culture media gave background levels of radioactivity (data not shown).

Analysis of virus-specific RNA in control and dbcAMP-treated cells

The transcription of virus-specific sequences in K-A31 cells, grown in medium with or without dbcAMP, was analysed by hybridizing cell RNAs with $^3$H-cDNA of Ki-MSV (Ki-MuLV). Results presented in Fig. 3 represent the pooled data from many experiments. Hybridization curves were plotted by a Hewlett Packard 9830A calculator plotter using kinetic parameters obtained from computer analysis (Pearson et al. 1977). The pooling of hybridization data obtained using RNA from cells harvested and extracted on different occasions introduces error into the determination of saturation values because of the influence of points from experiments reaching saturation at slightly different $R_0$ values; however, a good fit to the data was obtained in all cases [root mean square (RMS) of approx. 0.03].

Cytoplasmic RNA from untreated K-A31 cells hybridized 38% of input $^3$H-cDNA (Fig. 3a) while cytoplasmic RNA from dbcAMP-treated cells hybridized only 28% of input counts (Fig. 3c), showing that certain virus sequences were absent from the cytoplasm of treated cells. However, nuclear RNA from control (Fig. 3b) and treated (Fig. 3d) cells hybridized 38% and 43% of inputs respectively. This indicates that those sequences absent
### Table 1. Hybridization of various RNAs to Ki-MSV (Ki-MuLV)-specific $^3$H-cDNA*

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Normalized hybridization values</th>
<th>% Input counts hybridized</th>
<th>No. of expts</th>
<th>No. of points after saturation</th>
<th>Standard deviation of saturation values</th>
<th>Range of saturation values</th>
<th>Units of $R_{at}$ over which saturation was maintained</th>
<th>Maximum $R_{at}$ attained × 10^4</th>
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<tr>
<td>NIH/3T3 cells producing Ki-MSV (Ki-MuLV)</td>
<td>100</td>
<td>70</td>
<td>4</td>
<td>18</td>
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<td>7</td>
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<td>80</td>
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<td>2</td>
<td>7</td>
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<td>3</td>
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<td>22</td>
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<td>38</td>
<td>4</td>
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<td>$2.00$</td>
<td>6</td>
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<tr>
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<td>43</td>
<td>4</td>
<td>9</td>
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<td>7</td>
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<td>K-A31 nuclei + dbcAMP-treated K-A31 nuclei</td>
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<td>1</td>
<td>6</td>
<td>$1.72$</td>
<td>5</td>
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<tr>
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<td>56</td>
<td>39</td>
<td>1</td>
<td>5</td>
<td>$2.17$</td>
<td>5</td>
<td>$12,700$</td>
<td>$1.6$</td>
</tr>
<tr>
<td>Ki-MuLV producer cells + K-A31 cytoplasm</td>
<td>100</td>
<td>70</td>
<td>3</td>
<td>11</td>
<td>$3.22$</td>
<td>9</td>
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<tr>
<td>Ki-MuLV producer cells + dbcAMP-treated K-A31 cytoplasm</td>
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<td>3</td>
<td>13</td>
<td>$2.59$</td>
<td>7</td>
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<td>$1.10$</td>
<td>5</td>
<td>$27,600$</td>
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* Additive hybridizations were performed using saturating amounts of each RNA; values are shown minus background (4%).
Fig. 3. Detection of virus-specific RNA in control and dbcAMP-treated K-A31 cells. The results of hybridization analysis are expressed as a function of $R_{ot}$ (RNA concentration $\times$ time). The fraction of cDNA hybridized was calculated from $S_1$-resistant counts described in Methods and has been corrected for background (4%). All symbols represent experimental data and continuous lines are computer fits to the data (Pearson et al. 1977). (a) Cytoplasmic RNA from control cells; 39 points, RMS = 0.032. (b) Nuclear RNA from control cells; 28 points, RMS = 0.030. Dotted line represents corresponding cytoplasmic RNA. (c) Cytoplasmic RNA from dbcAMP-treated cells; 37 points, RMS = 0.025. (d) Nuclear RNA from dbcAMP-treated cells; 27 points, RMS = 0.031. Dotted line represents corresponding cytoplasmic RNA.

from the treated cell cytoplasm are transcribed and present in the nucleus. Comparison of Fig. 3(b) and 3(d) suggests that perhaps virus-specific RNA destined for the cytoplasm is processed differently in dbcAMP-treated cells. We do not consider the difference in saturation values chosen by the computer for the nuclear RNAs to be significant. The higher value for treated cell nuclear RNA was not obtained in additive hybridizations involving different batches of this RNA and may reflect the behaviour of a particular isolate at these very high $R_{ot}$ values.

$R_{ot}$ analysis (Leong et al. 1972) indicates that concentrations of virus RNA are slightly lower in treated than control nuclei, ($R_{ot}$ values of $1.1 \times 10^5$ and $8.5 \times 10^8$ respectively). This difference is small and we do not interpret this as a direct effect of dbcAMP on transcription. The concentration of virus sequences in treated cell cytoplasm ($R_{ot}$ of $7.0 \times 10^5$) is however, about half that of untreated controls ($R_{ot}$ of $3.5 \times 10^5$) and is perhaps another indication that movement of virus RNA from nucleus to cytoplasm is altered in dbcAMP-treated cells.

We cannot discount the possibility that certain virus-specific RNA sequences are rapidly degraded in the cytoplasm of treated cells but consider this explanation unlikely since with the methods of RNA extraction used and the high $R_{ot}$ values attained in hybridizations ($>2.5 \times 10^6$) we would expect to detect such sequences.
RNA from NIH/3T3 cells transformed and producing Ki-MSV (Ki-MuLV) hybridized 70% of the input 3H-cDNA and all other hybridization values have been normalized to this value and are shown in Table 1. The normalized value of 54% obtained using K-A31 cell RNA is in agreement with earlier observations (Benveniste & Scolnick, 1973; Benveniste et al. 1973). RNA from NIH/3T3 cells infected with and producing Ki-MuLV, hybridized the probe to a value of 80% suggesting that rat-specific sarcoma sequences should constitute the remaining 20%. RNA from AA-ascites rat tumour cells which are devoid of MuLV sequences but express all of the rat sequences contained in the Ki-MSV genome (Anderson & Robbins, 1976) hybridized the probe to 21%. The rat-specific and leukaemia-specific sequences within the probe are therefore complementary.

Since the probe contained both rat-specific and MuLV-specific cDNA transcripts, additive hybridization experiments were performed to characterize further the nuclear-restricted sequences in dbcAMP-treated cells. When RNA from NIH/3T3-Ki-MuLV producer cells was present in the hybridization reactions with RNA from treated or untreated cells, it served to bind homologous leukaemia sequences at low R<sub>o</sub>. The 3H-cDNA was then effectively a probe for the rat-specific sequences present in the cell RNAs. The cytoplasmic RNA from control cells in conjunction with the Ki-MuLV producer-cell RNA hybridized 100% of the probe and suggests complete transcription of the rat-specific sequences in K-A31 cells (Table 1). Treated cell cytoplasmic RNA plus Ki-MuLV producer-cell RNA hybridized to only 79%, a value approximately equal to that obtained with Ki-MuLV producer-cell RNA alone. When RNA from AA-ascites cells was mixed with treated cell cytoplasmic RNA the extent of hybridization achieved (57%) was similar to that achieved with untreated cell cytoplasmic RNA or either of the nuclear RNAs. The sequences absent from the cytoplasm of treated cells were therefore, quite clearly, rat-specific.

It might be argued that sequences present in treated cell nuclei over and above those in the cytoplasm could be additional leukaemia virus sequences, induced by the treatment and restricted to the nucleus, which coincidently raised the hybridization level to that obtained using RNA from untreated cells. Such a set of events would have required an explanation of the results based on transcriptional regulation of rat-specific sequences. This was shown not to be the case, however, by additive hybridizations involving treated cell nuclear RNA with either untreated cell nuclear RNA or AA-ascites cell RNA. In both cases values were 56%, similar to that of treated cell nuclear RNA alone (Table 1).

**hnRNA synthesis in dbcAMP-treated cells**

To ensure that the dbcAMP treatment was not adversely affecting DNA-like, RNA synthesis, we pulse-labelled cells for 10 min with 2-3H-adenosine and determined rates of hnRNA synthesis by monitoring the specific activities of ATP and RNA as detailed in Methods. The attraction of this technique is that since the rates of synthesis are determined using the specific activity of the ATP precursor pool, they are independent of any influence the dbcAMP treatment might have on the size of the pool or on membrane transport of the 3H-adenosine.

The combined results of two experiments, each of which comprised several determinations made after 24, 48 and 72 h growth in dbcAMP-containing medium, revealed that rates in treated cultures were 111 ± 3%, 109 ± 15% and 111 ± 13% of the control values respectively. This clearly demonstrates that hnRNA synthesis is not greatly reduced in treated cells. The fact that values are slightly higher than controls may be due to a slightly lower per cell level of ribosomal RNA in the slower growing cells, since specific activity for hnRNA was expressed as disintegrations/min in rapidly labelled RNA, divided by A<sub>260</sub> of total RNA. In a short pulse, label goes preferentially into hnRNA (Houssais & Attardi, 1966; Soeiro et al. 1968).
DISCUSSION

The results obtained from this study indicate very clearly that certain RNA species complementary to the rat-specific sequences of the Ki-MSV genome are absent from the cytoplasm of dbcAMP-treated K-A31 cells. Furthermore, the evidence suggests that some form of post-transcriptional processing is involved since these sequences are transcribed and are present in the nucleus in quantities which are easily detected. All, or nearly all, of the rat-specific sequences appear to be involved. It seems likely, therefore, that the portion specific for transformation, the src gene transcript, is restricted to the nucleus, especially considering the phenotypic response of the cells.

Cultures were maintained in medium containing dbcAMP and theophylline for 72 h to allow the development of homeostasis in terms of protein and RNA species and to optimize cell yields under conditions causing reduced growth rates. Medium was changed daily and butyrate has been eliminated as a possible intermediary. However, because of the long exposure time, the possibility remains that results may be due to secondary effects of the treatment rather than the direct influence of dbcAMP.

Considerable evidence now exists for the presence of sub-genomic virus-specific mRNAs in cells infected by murine mammary tumour virus (Robertson & Varmus, 1979) and by both avian (Mellon & Duesberg, 1977; Pawson et al. 1977; Weiss et al. 1977; Krzyzek et al. 1978; Parsons et al. 1978) and murine (Van Zaane et al. 1977; Fan & Verma, 1978) type-C viruses. These messages are probably derived from genomic-size mRNA by a processing step involving a shortening from the 5'-end with preservation and translocation of the 5'-terminus (Van Zaane et al. 1977; Krzyzek et al. 1978; Robertson & Varmus, 1979). Since it has been established that the rat-specific sequences of Ki-MSV are located primarily at the 5'-end of the genome (Shih et al. 1978a, b), our results can be explained by such a mechanism. One should, however, expect the absence of a Ki-MSV mRNA of a specific size from the cytoplasm of dbcAMP-treated cells and this question is currently under investigation in our laboratory.

Treatment of avian sarcoma virus-infected chick embryo fibroblasts with dbcAMP resulted in a fourfold reduction in virus RNA when monitored with genomic 3H-cDNA, but up to a tenfold reduction with src-specific 3H-cDNA and was interpreted as a suppression of transcription (Guntaka & Weiner, 1978). An increased rate of RNA turnover was not excluded however and, since total cell RNA was used, these data might also be interpreted as involving some form of RNA processing.

The transcription and restriction of globin-specific RNA to the nucleus of transformed erythroid cells has recently been reported (Therwath & Scherrer, 1978). The time course of the phenotypic and growth changes observed in dbcAMP-treated K-A31 cell cultures is compatible with a model involving the restriction of rat-specific information to the nucleus and gradual loss or dilution of pre-existing mRNA and proteins. The processing of mRNA is quite likely an important step in the expression of the transformed character and the direct or indirect effects of dbcAMP treatment may prove to be a useful tool in its investigation.

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