not known whether synthesis of RNA precedes the increase in antigen.

These results suggest that dexamethasone has a significant effect on MMTV expression in tissue culture cell lines of mammary origin. Viral RNA and antigens were increased as much as 20-fold over basal levels. In addition, a DNA polymerase similar to the type B viral reverse transcriptase was detected in culture fluid supernatants from a permanent cell line. If these particles contain 60*S* to 70*S* MMTV RNA, then a practical in vitro source of MMTV for immunological, biochemical, and biological application may be possible.

Halogenated pyrimidines induce type C viruses from a variety of murine cells (*16*), and dexamethasone was reported to stimulate production of the type C viruses induced by halogenated pyrimidines (*17*). Dexamethasone stimulation of type B particle production from the cell lines described here does not require treatment of cells with halogenated pyrimidines; these substances seem to have little effect with or without dexamethasone on type B particle production in the cell lines thus far examined. These results strengthen our observations that the expression of type B and type C viruses appear to be regulated independently in murine cells (*6*). Thus, in the type B virus system, with the use of a more natural inducer such as corticosteroids, it may be possible to study the natural mechanisms of cellular control of MMTV expression. If these controls involve transcriptional regulation, then this is an ideal system also for studying the mechanism of corticosteroid action by using [^{3}H]DNA • RNA hybridization to follow hormonal effects on RNA transcription from defined genes.

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16 October 1973; revised 3 December 1973 ■

Metabolic Differences between Normal and Neoplastic Cells: Effects of Aminonucleoside on Cytoplasmic Messenger RNA

Abstract. *Treatment of cultured normal human fibroblasts with an adenosine analog (aminonucleoside of puromycin) rapidly inhibits the appearance of cytoplasmic messenger RNA, identified by its polyadenylate sequence. Similar treatment of SV40-transformed fibroblasts does not lead to such an inhibition. Cordycepin, another analog of adenosine, inhibits polyadenylate-containing cytoplasmic RNA in both types of cell.*

When exposed to the adenosine analog aminonucleoside of puromycin, cultured human embryonic lung fibroblasts, WI38 cells (*1*), are arrested in the G1 (before DNA synthesis) and G2 (before mitosis) phases of the cell cycle. However, derivatives of these cells transformed by the oncogenic virus SV40 (WI38-VA13 cells) (*2*), and HeLa cells (a line originally cultured from a spontaneously arising carcinoma of the cervix) can traverse the cell cycle in the presence of this inhibitor (*3*). We have shown that treatment of normal WI38 cells with aminonucleoside inhibits the serum-induced stimulation of the synthesis of some classes of small-molecular-weight nonhistone nuclear proteins and the subsequent stimulation of DNA synthesis, while these processes in transformed WI38-VA13 cells are unaffected by aminonucleoside (*4*). In view of the evidence that nonhistone nuclear proteins are mediators of the stimulus to cell proliferation (*5*), the difference in the sensitivity to aminonucleoside between the growth of normal and transformed cells may lie in the lack of effect of this antimetabolite on the synthesis of small nonhistone nuclear proteins in the transformed cells.

Aminonucleoside has no direct inhibitory effect on protein synthesis as measured by the incorporation of [^{3}H]leucine into total cellular proteins (*3*). It inhibits RNA synthesis, principally ribosomal RNA synthesis, but this effect was evident in both normal and neoplastic cells (*6*). Therefore we have now studied the question of whether the appearance of messenger RNA (mRNA) in the cytoplasm is inhibited by aminonucleoside in normal human fibroblasts, but not in SV40-transformed fibroblasts. The effect of cordycepin (3′-deoxyadenosine), which is equally inhibitory to the growth of

normal and transformed cells, and is known to inhibit the synthesis of the polyadenylate [poly(A)] segment of mRNA (*7*), was also compared in these cells.

Exponentially growing monolayers of WI38 and of WI38-VA13 cells (7×10^6) were fed with fresh medium containing 10 percent serum 18 hours before the start of the experiment. The inhibitors—aminonucleoside (100 μg/ml) or cordycepin (20 μg/ml) were added in fresh medium, and after 60 minutes [^{3}H]uridine (New England Nuclear, specific activity, 27.3 c/mmole) was added to all cultures to a final concentration of 5 μc/ml for an additional 30 minutes to allow incorporation of the isotope. The medium was removed, and the cell sheet was placed on ice and washed three times with Earle's balanced saline; the cells were then harvested. Nuclei and cytoplasm were separated as described by Penman (*8*). The RNA from the cytoplasm was extracted by the hot phenol–sodium dodecyl sulfate (SDS) method (*8, 9*), and precipitated with 2.5 volumes of 95 percent ethanol at − 20°C for 60 minutes. After centrifugation (15,000 rev/min for 45 minutes; JA20 rotor of Beckman intermediate centrifuge), the pellet was resuspended in 5 ml of SDS buffer (0.5 percent SDS, 0.1*M* NaCl, 0.01*M* tris-HCl, *p*H 7.4), and the material was passed through glass filters (Whatman, GF, 24 mm) impregnated with polyuridylic acid [poly(U)]; the flow rate was 1 ml/min. The filters were impregnated by a modification of the method of Sheldon *et al.* (*10*), by applying 0.2 ml of a solution of poly(U) in water (1.5 mg/ml). The material bound to poly(U) filters was eluted with a formamide-buffer mixture (50 percent formamide in 0.01*M* tris-HCl, *p*H 7.4, 0.5 percent SDS). Less than 1 percent of poly(U)-bound radioactivity remained on the filters after the elution. Carrier RNA (yeast transfer RNA, final concentration 400 μg/ml) and NaCl (final concentration 0.1*M*) were added to the eluates, and the RNA was precipitated with 2.5 volumes of 95 percent alcohol at − 20°C for 60 minutes. The RNA was sedimented, and the pellet was resuspended in 1 ml of SDS-buffer, and overlayered on a linear sucrose gradient (15 to 30 percent weight by volume) containing SDS buffer. The 30-ml gradients were centrifuged at 25,000 rev/min for 18 hours (Spinco SW 25.1 rotor). Fractions (24 drops) were collected from the bottom of the gradients and precipitated with 10 ml of 0.2*N* perchloric acid; the radioactivity in the precipitate was measured in a Mark I Nuclear Chicago Scintillation Counter.

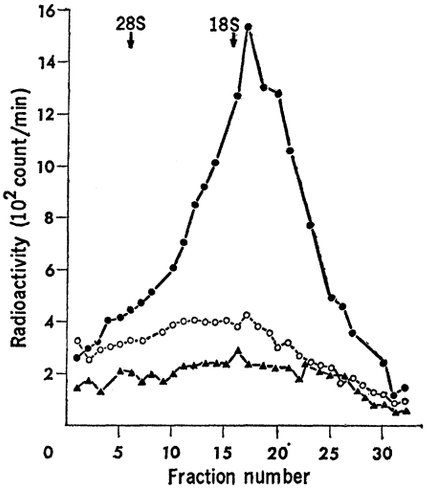

Fig. 1. Effects of aminonucleoside and cordycepin on the binding of cytoplasmic RNA from WI38 cells to filters impregnated with polyuridylic acid. Arrows indicate the peaks of distribution of ribosomal RNA species prepared from HeLa cells. ●, Control cells exposed to fresh medium only; ○, cells exposed to fresh medium containing aminonucleoside (100 μg/ml); ▲, cells exposed to fresh medium containing cordycepin (20 μg/ml).

The distribution of radioactivity in the gradients is shown in Fig. 1 for WI38 cells, and in Fig. 2 for WI38-VA13 cells. The poly(A)-containing RNA from control cultures shows the distribution expected of cytoplasmic mRNA (*11*), but the appearance of RNA of this type in the cytoplasm is virtually suppressed by exposure to cordycepin for 60 minutes in both types of cell. The effect of aminonucleoside, however, is clearly selective. Little mRNA can be detected in the cytoplasm of normal cells treated with aminonucleoside, while the amount of cytoplasmic mRNA is unaffected by this treatment of transformed cells. The shift toward higher molecular weights in the population of mRNA's from the cytoplasm of aminonucleoside-treated transformed cells was noted in four consecutive experiments, but is at present unexplained.

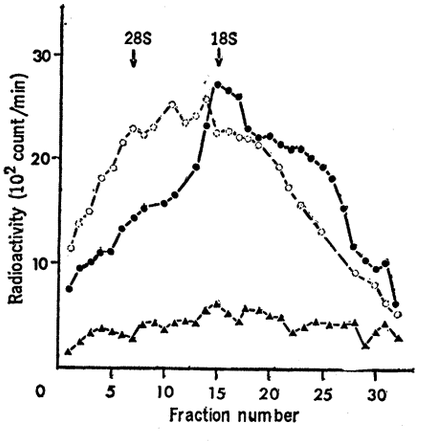

Fig. 2. Effects of aminonucleoside and cordycepin on the binding of cytoplasmic RNA from WI38-VA13 cells to poly(U)-impregnated filters. Experimental details as for Fig. 1.

The findings indicate that a step in the sequence of mRNA synthesis, processing and transport to or stabilization in the cytoplasm may be altered in the neoplastic cells, since it is apparently not greatly affected by aminonucleoside. Measurement of intracellular concentrations of aminonucleoside in cells exposed to the tritiated form of this compound has shown that aminonucleoside enters the transformed cells at about the same rate as it enters the normal cells (*3*), and in other experiments it was shown to inhibit ribosomal RNA synthesis in both types of cell with approximately the same kinetics (*6*); hence there is no reason to suppose that it is the inhibitor that is processed into its metabolically active form differently by the transformed cells.

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18 October 1973