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Relationship of Friend Murine Leukemia Virus Production to Growth and Hemoglobin Synthesis in Cultured Erythroleukemia Cells

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The factors that control oncornavirus formation were analyzed in Friend leukemia cells that undergo hematopoiesis when treated with dimethyl sulfoxide. Suspension cultures of Ostertag FSD-1 cell line were found to enter a G_0 or resting state at the end of their proliferative phase and to simultaneously cease producing helper and dependent components of Friend virus. Whereas the decline in virus production is at least 100-fold, rates of cellular RNA and protein synthesis are only slightly lower in resting than in growing cells. Both resting and growing cells contain similarly large concentrations of the viral proteins P(30) and P(12). Dimethyl sulfoxide induces hemoglobin synthesis in growing cells, but its effects on virus production appear to be indirect results of its action to inhibit cell growth and thus to delay entry of cells into the G_0 resting state. Furthermore, variant cell lines were obtained with differing abilities to synthesize virus or hemoglobin. Some lines no longer produce infectious virus, although they all harbor murine leukemia virus genes which are expressed to varying extents. The major internal protein of these oncornaviruses, P(30), is synthesized in large amounts by all of the cell lines. These results suggest that Friend virus production is not coinduced with erythroid differentiation, as had been proposed, but rather is controlled by a cellular growth cycle.

Permanent lines of leukemia cells have been isolated from mice infected with Friend virus (10, 28, 38). These Friend leukemia (FL) cells grow in suspension with a doubling time of 18 to 24 h and synthesize hemoglobin when treated with 1 to 2% dimethyl sulfoxide (Me_2SO). With this inducer as well as most others (25, 40), at least two cell divisions precede appreciable synthesis of hemoglobin (25) or of hemoglobin mRNA (31). Because of this requirement for cell division, induction of hemoglobin synthesis has been routinely accomplished by diluting concentrated suspensions of FL cells into fresh medium containing Me_2SO (10, 18, 25, 28, 31, 38). Therefore, as described below, effects of Me_2SO or of hematopoiesis must be carefully distinguished from those due only to fresh medium or to cellular proliferation.

Electron microscopic comparisons of Me_2SO treated with control FL cultures have indicated that induced cells contain greatly increased numbers of budding virus particles on their surface and intracellular membranes and in intracytoplasmic vacuoles (16, 27, 33, 38). In addition, secretion of Friend virus increases 10- to 100-fold when certain FL cell lines are induced to differentiate by treatment with 1% Me_2SO (8, 27, 39). These studies have indicated that oncornavirus synthesis and hematopoiesis in FL cells may be coinduced, although studies with interferon have shown that completion of virus synthesis is not a prerequisite for hemoglobin formation (39). Coupling of cellular differentiation with virus synthesis has also been observed for other cells (11, 14, 37, 43) and has led to the proposal that differentiation may be a major regulator of oncornavirus synthesis during the mammalian life cycle (4).

As a model for analyis of factors controlling oncornavirus metabolism, we have studied Friend virus synthesis in FL cells. Our results show that virus production is controlled by a cellular growth cycle rather than by hematopoiesis. Me_2SO perturbs this cellular cycle and thereby can alter virus formation indirectly.

MATERIALS AND METHODS

Cells and their culture. Friend clone 745 was obtained from the Genetic Mutant Cell Culture Repository. Lines FSD-1, F4N (a clone of FSD-1), and F4-6 (a subclone of F4N) were generously supplied by W. Ostertag. Suspension cultures of these lines have been transferred and maintained in our laboratory since their arrivals, and, in recognition of

possible changes they may have undergone, we have added the suffix /K to their designation. We isolated clones from FSD-1/K by diluting a stationary-phase culture (2×10^6 to 3×10^6 cells/ml) to 1.5 cells/ml with fresh medium and plating 0.2 ml of culture fluid in each well of a Falcon MicroTest II tissue culture plate. The maintenance conditions for our cell lines are described in detail elsewhere (18). The doubling time is generally 18 to 24 h. For induction of hemoglobin by Me_2SO, successive 1:1 dilutions of a stationary-phase culture with fresh medium were made at 0, 24, and 48 h. At these times, the induced cultures were made successively 1, 1.5, and 2% in Me_2SO. In other experiments, Me_2SO concentrations were kept at 1 or 1.5% throughout.

Virus assays. SFFV. The titer of dependent spleen focus-forming virus (SFFV) was determined in 6- to 8-week-old DBA/2 male mice by the macroscopic spleen focus assay of Axelrad and Steeves (2). The disease induced by the SFFV from different cell lines showed considerable variation. In some cases no spleen lesions were observed at 9 days but were evident after a longer period of infection. In other cases visible foci were too small to be scorable by the criteria of the assay. Therefore, a titer of zero in this assay does not necessarily reflect a complete absence of SFFV. Where indicated, SFFV titers were evaluated by the spleen weight assay of Rowe and Brodsky (32). Changes in the spleen weight 13 days after infection were expressed as the percentage of change in the ratio of spleen weight to body weight determined by comparison to an average ratio obtained from a large number of uninfected animals. All SFFV assays were done on at least three mice.

LLV. The titer of lymphatic leukemia virus (LLV) was measured using a variation of the "S^+L^-" method of Bassin et al. (6). The S^+L^- cells were kindly provided by P. J. Fischinger, National Institutes of Health. Briefly, 2×10^5 S^+L^- cells were plated in a 25-cm^2 tissue culture flask containing 5 ml of McCoy 5a medium plus 10% fetal calf serum. After 24 h of incubation in 5% CO_2 at 37 C, the medium was removed and replaced with 1 ml of medium containing 25 μg of DEAE-dextran (Sigma) per ml for 30 min at 37 C. After decanting the dextran, 0.5 ml of virus sample was added, and the flask was incubated for another 30 min. Five milliliters of fresh medium was then added and the flasks were incubated, tightly closed, at 37 C. After 3 days, an additional 3 ml of medium was added. Foci were counted 6 days after sample application. The half-life of LLV in the culture fluid was determined by removing F4-6/K cells by centrifugation, filtering through a 0.22-μm membrane filter (Millipore Corp.), and incubating at 37 C in 5% CO_2. Samples were removed at 0, 2, 4, and 8 h and assayed for LLV by the S^+L^- assay.

Reverse transcriptase assay. Virus was precipitated from culture medium with concanavalin A (36), and the virus pellet was disrupted in 25 μl of 0.1% Triton X-100 (Sigma) and incubated at 37 C for 10 min. The solution was adjusted to a final volume of 100 μl containing 50 mM Tris-hydrochloride, pH 8.3, 6 mM $MgCl_2$, 1 mM dithiothreitol, 60 mM NaCl, 158 μM [^{3}H]dGTP (25 counts/min per pmol), 10 μg of poly(rC) (Collaborative Research, Waltham, Mass.), and 5 μg of oligo(dG_{12-18}) per ml (Collaborative Research). The reaction was allowed to proceed at 37 C for 15 min and was terminated by the addition of 0.5 ml of 0.1 M sodium pyrophosphate, 100 μg of *Escherichia coli* tRNA, and 0.5 ml of 25% trichloroacetic acid. Samples were collected on glass fiber filters and counted in toluene-based scintillation fluid according to the procedure described by Birnboim (7).

Immunological methods. P(30), the major internal polypeptide of the virion, was generally measured by the microtiter complement fixation method (26). P(30) and P(12) were also assayed by a modification of the competition radioimmunoassay of Stephenson et al. (35). Growing and stationary F4-6/K cells were collected by centrifugation and disrupted with PE buffer (10 mM potassium phosphate, pH 7.8; 10 mM EDTA; 0.6% bovine serum albumin) plus 0.5% Triton X-100 in a glass homogenizer with Teflon pestle. Serial dilutions of the lysate were incubated for 1 h at 37 C with a limiting amount of the appropriate goat monospecific antiserum (enough to precipitate 50% of the ^{125}I-labeled antigen added) in a reaction mixture consisting of PE buffer and 0.1% normal goat serum, in a total of 0.160 ml. ^{125}I-labeled antigen (10,000 counts/min) in 0.020 ml was added, and incubation was continued for a further 3 h at 37 C. A 0.020-ml amount of undiluted rabbit anti-goat serum was added to each reaction mixture, and samples were incubated overnight at 4 C. Immunoprecipitates were collected by centrifugation (2,000 $\times$ *g* for 30 min at 4 C), and the radioactivity in the pellets was measured in a Beckman Biogamma counter. Antiserum to Rauscher leukemia virus P(30) was generously donated by R. V. Gilden, Flow Laboratories. Antiserum to Rauscher P(12) and ^{125}I-labeled P(30) and P(12) were generously donated by S. R. Tronick, Viral Carcinogenesis Branch, National Cancer Institute.

DNA synthesis. DNA synthesis was measured by the incorporation of [^{3}H]thymidine into trichloroacetic acid-precipitable material. Cell cultures were incubated at a concentration of 10^6 cells/ml for 30 min at 37 C with 0.5 μM [^{3}H]thymidine (2×10^4 Ci/mol). At 0 and 30 min, 50- and 100-μl aliquots of the cell culture fluid were placed on 24-mm glass fiber filters. The filters were dried, rinsed (cold 10% trichloroacetic acid twice, cold 5% trichloroacetic acid twice, ethanol, and ether) to remove unincorporated label only, and the radioactivity remaining on the filter was solubilized for 1 h in 0.5 ml of toluene-protosol (New England Nuclear) (2:1). Radioactivity was measured in 5 ml of toluene containing 0.3% PPO (2,5-diphenyloxazole), 0.03% POPOP [1,4-bis-(5-phenyloxazolyl)benzene], and 0.1% acetic acid in a liquid scintillation spectrometer.

Other methods. Protein concentrations were assayed by the method of Lowry et al. (24). Hemoglobin concentrations were measured in FL cell lysates from the visible absorption spectra (18). Acridine orange-stained FL cells were quantitatively analyzed for green fluorescence due to DNA in a cytofluorograph (1). Data for 30,000 cells were accumulated and plotted on an oscilloscope.

RESULTS

A basic relationship between cellular growth and virus production. Hemoglobin synthesis is induced by diluting stationary-phase FL cultures into fresh medium which contains Me_2SO. Normally, the cultures are diluted twofold at 0, 24, and 48 h and hemoglobin is assayed at 72 or 96 h. This protocol is widely used and is optimal for hemoglobin induction and for cell viability, presumably because a high cell concentration is maintained (approximately 10^6 cells/ml). Me_2SO can harm cell viability and slow growth rate, especially if the cells are highly diluted (10, 16).

Figure 1 shows the growth rate and virus (LLV and SFFV) production characteristics of F4-6/K cells maintained according to the above dilution protocol, both in the presence and absence of Me_2SO. The same pattern is found in all of the cultures. The concentration of LLV rises while the cells are growing and declines when the cells approach the stationary phase. The control culture becomes stationary, and its titer of LLV drops before these same changes occur in the Me_2SO-treated cultures, presumably because Me_2SO slows cellular growth rate (Fig. 1A and B). For example, growth is so inhibited in 1.5% Me_2SO that the cells do not reach stationary phase by 96 h, and LLV concentration remains elevated. All of this data is consistent with the hypothesis that LLV is produced only by growing cells. This apparent relationship between cell growth and virus production was also observed when our other cell lines were analyzed.

Although the maximum concentration of SFFV appears to correlate somewhat with the extent of hemoglobin production (see Fig. 1 legend), the variation inherent in the SFFV assay (32) does not allow one to interpret more than a general pattern of rise and decline in SFFV production similar to that of the LLV. This pattern was observed in all SFFV-producing lines examined, regardless of their ability to produce hemoglobin. Since SFFV is a dependent virus (23), the pattern of SFFV secretion may be a consequence of its dependency upon LLV.

The basic pattern of virus release observed in FL cultures diluted according to the hemoglobin induction protocol (i.e., Fig. 1) is also observed when FL cells at their stationary phase are diluted to a low concentration (2×10^5 cells/ml) and are simply allowed to grow toward saturation. In this case, as seen in Fig. 2, LLV and SFFV titers in the culture media are approximately 100-fold higher in rapidly growing cells (48 to 72 h), as compared with stationary-phase cells (96 h).

Virus concentrations measured in Fig. 1 and 2 are dependent both on rates of release from cells and on rates of inactivation in the culture medium. Rates of virus inactivation were measured at 37 C in conditioned tissue culture medium from control and Me_2SO-treated cultures. The half-life of LLV in media containing Me_2SO was identical with that in control media in every case. In conditioned medium from stationary-phase FL cultures, LLV is inactivated with a half-life of 2.3 h, whereas the half-life is 3.7 h in medium from rapidly growing cultures and is 4.8 h in fresh medium. These rates of spontaneous virus inactivation are similar to rates reported previously for oncornaviruses and are very rapid compared with the 24-h intervals between sampling points in Fig. 1 and 2. Therefore, the data in these figures must reflect the rates of virus release from cells at the times indicated. In addition, we have established that the presence of 50% conditioned medium from stationary-phase cultures does not significantly inhibit virus production by growing FL cells during a 24-h period (data not shown). Therefore, secretion of an inhibitor is unlikely to account for the observed patterns of virus production.

The virus released from growing F4-6/K cells was found to be N-tropic, as determined by the spleen focus assay in DBA/2 compared with BALB/c mice. This is of some interest because the virus originally used to produce the leukemia was NB-tropic (8).

Characterization of the cellular growth cycle. Although the concentration of FL cells in culture medium approaches a "stationary value" at 2×10^6 to 3×10^6 cells/ml, this value could reflect entry of cells into a G_0 resting state, blockage at some point in the mitotic cycle, or a dynamic balance between continuing mitosis and cell death. Analysis of acridine orange-stained cells, using a cytofluorograph to quantitatively measure the green fluorescence due to DNA (Fig. 3), has shown that FL cells in the stationary cultures are arrested in either a G_0 state or in the G_1 phase. The cells in the stationary cultures all (99%) contained the normal diploid amount of DNA (18), and cells with more DNA did not accumulate when these cultures were incubated with colchicine. On the contrary, approximately 30% of the cells in the growing FL cell culture contained more than the diploid quantity of DNA. As expected, colchicine treatment of this population caused an accumulation of cells having more DNA. In agreement with these conclusions, cells in me-

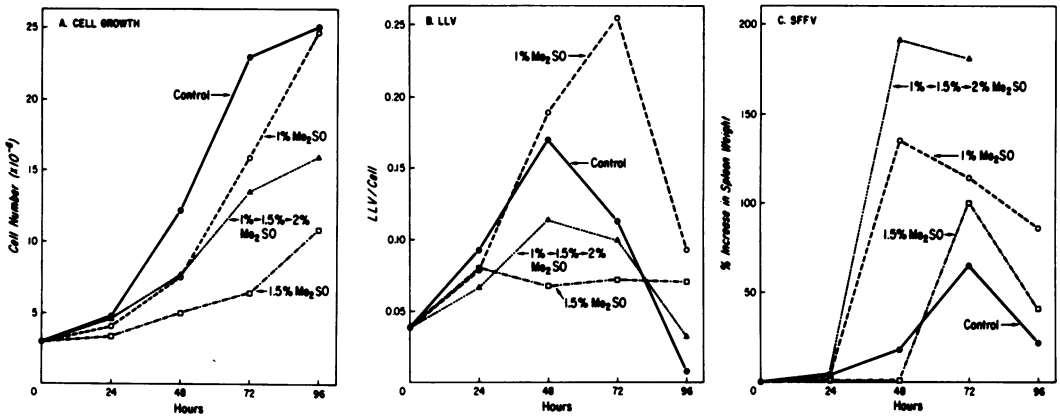

FIG. 1. *Growth and virus production of control and Me_2SO-treated F4-6/K cells were treated according to the hemoglobin induction protocol. The control received no Me_2SO. The treated cultures were kept at 1% Me_2SO or 1.5% Me_2SO throughout or were in 1% Me_2SO (0 to 24 h), 1.5% (24 to 48 h), and 2% Me_2SO (48 to 96 h). Hemoglobin concentrations were measured at 96 h and were negligible (<0.1% total cellular protein) in the control culture, 1.5% of the protein in that treated with 1% Me_2SO, 3.8% in the 1.5% Me_2SO-treated culture, and 5.3% in the 1% → 1.5% → 2% Me_2SO-treated culture. (A) Cell growth. The number of cells obtained from 1 original ml of an approximately stationary-phase culture is shown. Dilutions (1:1) with fresh medium were made after the cell counts were taken at 0, 24, and 48 h, so that the actual volume represented by the cell count is 1 ml at 0 h, 2 ml at 24 h, 4 ml at 48 h, and 8 ml at 72 and 96 h. (B) LLV production. The value shown is the number of LLV in the average volume of culture fluid containing one cell. (C) SFFV production. SFFV in the medium was assayed by the spleen weight method. The values plotted represent the spleen weight titer from a volume of culture fluid containing one cell. The SFFV assay at 96 h in the culture treated with 1% → 1.5% → 2% Me_2SO was unsuccessful.*

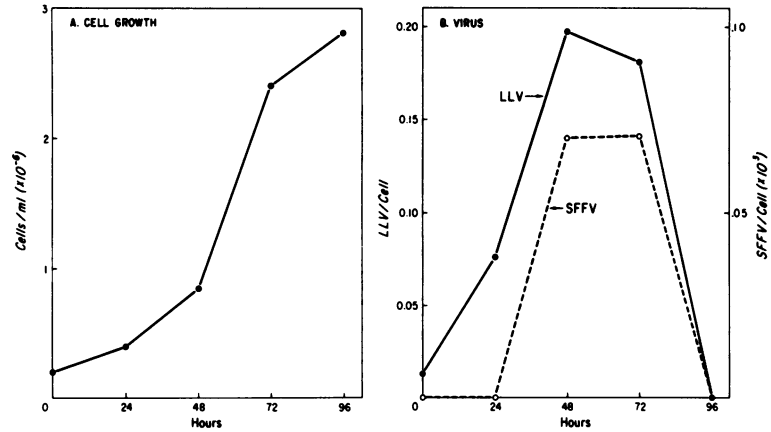

FIG. 2. *Growth and virus production of F4-6/K cells during a normal culture transfer. At 0 h a stationary-phase culture of cells was diluted to a concentration of 0.2 × 10^6 cells/ml with fresh medium. (A) Growth of cells. (B) Virus production. The LLV concentration shown is the titer of LLV from the volume of culture fluid containing one cell. SFFV in the culture fluid was measured by the spleen focus assay evaluated 9 days after infection.*

taphase were absent in the stationary cultures 12 h after exposure to colchicine, whereas at this time the growing culture contained 50 to 60% metaphase cells.

The cellular proliferation cycle was also analyzed by [^{3}H]thymidine incorporation. Stationary-cell cultures did not incorporate [^{3}H]-thymidine into DNA (Table 2). Furthermore, when such cultures were diluted into fresh medium, a 12-h lag phase preceded a synchronous wave of [^{3}H]thymidine incorporation. A peak of mitosis followed cell dilution by 18 h and the cell concentration doubled at this same time. Two waves of virus release preceded the first period of mitosis, but the major increase of virus production did not occur until the second

TABLE 1. *Half-life of biologically active LLV in the culture fluid of control and Me_2SO-treated cultures*

Hours after beginning induction protocol[a]	LLV half-life (h)[b]	
	Control	1% Me_2SO treated
0	4.8	
24	4.3	4.2
48	3.7	3.7
72	3.2	3.2
96	2.3	2.4

[a] Cultures were diluted 1:1 with fresh medium (±1% Me_2SO) after 0, 24, and 48 h as described in Materials and Methods.

[b] The time required for LLV to lose half their infectivity when incubated in conditioned culture fluid at 37 C was determined as described in Materials and Methods.

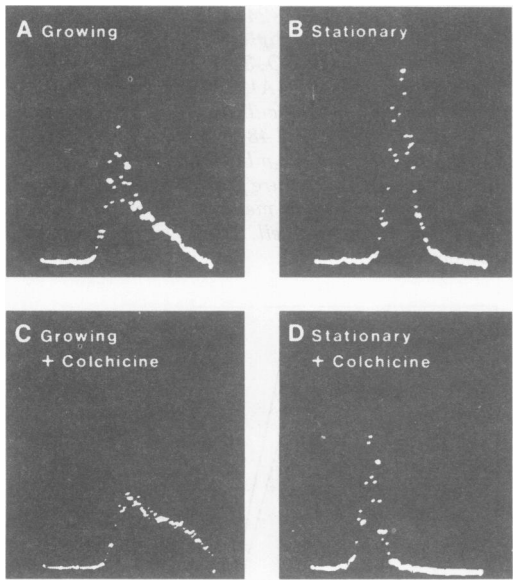

FIG. 3. *DNA content of cell populations as measured by acridine orange fluorescence. Cultures of growing- and stationary-phase F4-6/K cells were analyzed. Portions of each culture were treated with 0.01 μg of colchicine per ml for 4.5 h before assay. The abscissa is proportional to quantity of DNA and the ordinate to numbers of cells. (A) Growing culture; (B) stationary culture; (C) growing culture treated with colchicine; (D) stationary culture treated with colchicine. In the latter case, the ordinate scale was reduced by a factor of 2 to retain the entire curve on the oscilloscope. A total of 30,000 cells of each culture was analyzed.*

and third mitotic cycles. These observations support the conclusion that the stationary cells are arrested in a G_0 resting state; dilution of the cell culture causes a release from the arrest, and traverse through G_1 precedes the onset of DNA synthesis. Furthermore, virus production begins when the cells have reentered the mitotic cycle.

Presence of viral proteins in nongrowing cultures. The cessation of virus production in G_0 resting-state cells could be a consequence of several factors. However, it does not appear to be caused by a depletion of all viral structural proteins. The major viral proteins, P(30) and P(12), are present in similar concentrations in growing and resting F4-6/K cell cultures (Table 2).

Analysis of hematopoiesis and virus production in variant cell lines. As outlined in Table 3, we have isolated FL cell lines which have characteristic differences in hemoglobin and virus synthesis. Several of the lines derived for Ostertag's FSD-1 line have largely or completely stopped producing active virus, yet have retained ability to synthesize hemoglobin. One clone from this line (clone 31) makes only barely detectable hemoglobin (ca. 0.5% of its total protein). Friend's cell line 745 secretes barely detectable amounts of both viruses, although it makes a large amount of hemoglobin (up to 10% of its total protein).

Despite varying abilities to produce active virus, all of these cell lines habor murine leukemia virus genes which are expressed to varying extents (Table 3). For example, they all contain high titers of P(30), the major internal protein of the murine leukemia viruses. In addition, all the cell lines secrete while growing at least small amounts of particles which are precipitated by concanavalin A and which contain P(30) and reverse transcriptase. Apparently, these cell lines have retained virus which has spontaneously become defective in varying

TABLE 2. *DNA synthesis and the relative content[a] of two virion proteins in growing and stationary cells*

Cells	[^{3}H]thymidine incorporation (counts/min per 0.5 h per 10^3 cells)	Relative protein content[a,b]	
		P(30)	P(12)
Growing[c]	507	1.0	1.0
Stationary[c]	9	1.2	0.6

[a] Lysates of 10^7 growing or stationary cells were examined for their ability to compete with ^{125}I-labeled P(30) and P(12) in the radioimmune precipitation assay, as described in Materials and Methods. The dilution of growing cell lysate required to produce 50% inhibition of precipitation of the ^{125}I-labeled protein was arbitrarily set at 1.0.

[b] The P(30) assay was calibrated with a P(30) sample of known concentration. A value of 1.0 in the table corresponds to 0.7 pg of this protein per cell.

[c] Growing cells were harvested at a stage comparable to 48 h in Fig. 2A, and stationary cells were harvested at 96 h in Fig. 2A.

TABLE 3. *Properties of representative FL cell lines*

Cell line[a]	Hemoglobin synthesis[b]	LLV[c]	SFFV[c]	Cellular P(30)[d]	Culture fluid P(30)[e]	Culture fluid reverse transcriptase[e]
F4-6/K	+++	39,700	834	1,100	53	91
F4-N/K	+	20,500	0[f]	1,600	57	9.1
FSD-1/K	++	29	0	400	17	7.1
FSD-1/K (C 3)	+++	0	0	200	16	0.88
FSD-1/K (C 20)	+	361	11	500	31	6.1
FSD-1/K (C 28)	++	5	0	800	12	4.6
FSD-1/K (C 31)	±	0	0	800	11	2.9
Friend 745/K	++++	7	1	600	12	8.3

[a] Origin of the cell lines is described in Materials and Methods.

[b] Hemoglobin was induced as described in Materials and Methods and was assayed at 96 h. A score of + + + + indicates that approximately 8% of the total protein at 96 h is hemoglobin. The exact quantity of hemoglobin depends on the batch of fetal calf serum. Clone 31 was only weakly positive and contained approximately 0.5% hemoglobin.

[c] Cultures for assay of viral components were all started at 3×10^5 cells/ml and were harvested in the exponential phase after 48 h of growth. LLV and SFFV titers are given in plaques or foci produced from the volume of culture fluid which contained 10^6 of the growing FL cells. SFFV was measured by the spleen focus assay read on day 9 (see Materials and Methods).

[d] The units are complement-fixing titers per 0.025 ml of sedimented FL cells. On this scale, normal spleen cells are zero and leukemic spleen cells from FV-infected mice are approximately 400.

[e] A volume of culture fluid containing 10^6 cells was sedimented to remove cells and virus and was then precipitated with concanavalin A as described in Materials and Methods. The resulting precipitates were assayed for P(30) by complement fixation and for reverse transcriptase. Reverse transcriptase units are picamoles above background per 15 min of incorporation.

[f] This cell line made no spleen foci visible on day 9 of the assay; however, moderate splenomegaly was observed, and by day 13 infected mice had grossly enlarged spleens with confluent foci.

ways and to different extents. Nevertheless, expression of virus genes has not been suppressed completely in any cell line.

DISCUSSION

Release of active LLV and SFFV from FL cells correlates closely with a cell proliferation cycle. When cell cultures grow to a concentration of 2×10^6 to 3×10^6 cells/ml, mitosis ceases and the cells enter a G_0 resting state of proliferative arrest, as defined by Baserga (5). Unlike G_1 or other phases of the mitotic cycle, the G_0 state has an indefinite duration and is not preparatory to cell division. When diluted into fresh medium, FL cells synchronously leave the G_0 resting state and subsequently begin to release virus in greatly increased amounts. Furthermore, virus release stops when the cells again reach saturation (Fig. 1 and 2). That the major positive effect of dilution is related to cellular growth rather than simply to freshness of medium is suggested by the observation that Me_2SO concentrations which inhibit growth also inhibit LLV formation (Fig. 1). Furthermore, factors that inactivate virus rapidly or that inhibit virus production by growing cells have not been detected in culture medium from stationary-phase cells. In preliminary experiments, we have also found that the major burst of virus secretion after dilution can be blocked by inhibitors of DNA synthesis. Therefore, release from the repression of the G_0 state may require passage of the cells at least into *S* phase. The fact that virus production is greater during the second and subsequent mitotic cycles than during the first also suggests that full derepression of virus formation may require a major change in the intracellular milieu.

G_0 resting state cells are known to be active in macromolecular metabolism, although there are selective changes in synthesis of specific substances which distinguish them from growing cells. We have shown elsewhere that dilution of G_0 resting state FL cells into fresh medium is followed over the next 2 to 5 h by twofold increases in rates of RNA and protein synthesis (34). Both 4*S* and ribosomal RNA synthesis rates are enhanced, as is the rate of 45*S* ribosomal RNA precursor processing. Similar changes occur when fibroblast cultures are released from "density-dependent" growth inhibition (17) and in cultured WIL_2 lymphocytes (22). In the latter case, immunoglobulin synthesis requires cell growth. Although it has been reported that FL cell hemoglobin synthesis stimulated by several inducers requires cell proliferation (25, 40), a recent report indicates that butyric acid can induce hemoglobin synthesis in one cell line in the absence of mitosis

(20). However, butyric acid does not induce hemoglobin synthesis in our cell lines.

A relationship has been previously observed between oncornavirus synthesis and cellular growth in fibroblast cultures (3, 9, 11–13, 15, 21, 29, 30, 41, 42). For example, productive infection by Rous sarcoma virus is accomplished only in dividing cells (13, 42). Once infection is established, however, there are conflicting reports about whether continued Rous sarcoma virus secretion requires continued cell growth (15, 21). In addition, activation of synthesis of endogenous murine leukemia viruses by halogenated pyrimidines or inhibitors of protein synthesis also requires cellular growth (12, 41). Furthermore, Kirsten leukemia virus is released from chronically infected fibroblasts mainly during mitosis (29). Our data suggest that control of oncornavirus production by the cellular growth cycle occurs also in leukemic cells growing in suspension and may therefore be a general mode of control over these viruses. Since FL cells were obtained from leukemic mice, it also seems likely that this cellular control operates in cancer cells transformed in vivo.

The mechanism by which this control is exerted remains unknown. However, Paskind et al. (30) recently reported that viral RNA is not the rate-limiting component in virus production by G_0-arrested fibroblasts transformed with Moloney murine leukemia virus. Furthermore, they suggested that a cellular or viral protein might be the rate-limiting factor. Our experiments suggest that two of the major viral structural proteins, P(30) and P(12), are present in similar concentrations in growing and G_0-arrested FL cells. We are currently analyzing this aspect of the problem in greater detail.

These results raise serious questions concerning earlier correlations made between oncornavirus secretion and induction of hemoglobin synthesis in FL cells by Me_2SO (8, 16). It appears that in at least some of the previous studies "uninduced" cells at or near the stationary phase of their growth were used as controls for comparison with "induced" FL cells actively growing in 1 to 2% Me_2SO, whereas proper controls would have included analysis of uninduced FL cells at times throughout their growth cycle. Thus, the burst of infectious virus production after renewed cell growth has been interpreted as being linked to hematopoiesis. Our results show that a similar burst of virus production occurs in the absence of Me_2SO and is related to reentry of stationary-phase cells into the mitotic cycle. Furthermore, the tropism of the virus produced by growing cells is the same as that found by Dube et al. (8) in growing cells treated with Me_2SO. This supports our inference that the virus which the latter workers believed to have been induced by Me_2SO is in fact produced by growing cells in the absence of this compound. On the other hand, our results do not exclude the possibility that expression of one or more viral genes occurs coordinately with hematopoiesis. However, such gene expression is not rate limiting for LLV or SFFV secretion in our cell lines. In addition, it is possible that defective Friend virus or endogenous virus which we have not assayed do accumulate within FL cells during hematopoiesis in the manner suggested by the ultrastructural studies (16, 27, 33, 38). Nevertheless, this study indicates that hematopoiesis may have no effect on Friend virus release from FL cells and that Me_2SO at certain concentrations perturbs the cellular growth cycle and thereby may alter virus production indirectly.

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