recombination of the two parental chromosome sets. Recombination of electrophoretic phenotypes in *R. esculenta* inter-se crosses has been previously reported by these authors[29,30]. A recombination rate of 2–3% for the R-E system has been estimated by Uzzell et al.[31] from a similar study of 13 crosses involving *R. esculenta* males. Further cytological and biochemical experiments by using specific chromosomal markers and more specific antisera are needed to clarify the situation.

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Reversibility of the transformed and neoplastic phenotype. III. Long-term treatment with electrophoretically pure mouse interferon leads to the progressive reversion of the phenotype of X-ray transformed C3H/10T1/2 cells

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Summary. Electrophoretically pure mouse interferon induced the progressive reversion of the transformed phenotype of a clone of X-ray transformed C3H/10T1/2 cells. Cells of this clone did not harbor C-type particles and reverse transcriptase activity was not detected. Interferon-treated transformed cells were aligned without cellular overlapping and attained low cell densities. Morphologic changes were associated with the

appearance of a thick layer of submembranous microfilaments. The tumorigenicity of interferon-treated cells was markedly reduced. Back reversion to the transformed phenotype occurred progressively when these cells were passaged in the absence of interferon. These results suggest that interferon may induce the reversion of the transformed phenotype by a mechanism other than by its antiviral activity.

Introduction

We have previously reported that partially purified mouse interferon induced the progressive reversion of the transformed and malignant phenotype of X-ray-transformed C3H/10T1/2 cells[2]. Numerous C-type particles were observed in the untreated transformed cells and significant levels of reverse transcriptase activity were demonstrable in the cell free nutrient medium. Neither C-type particles nor reverse transcriptase activity was demonstrable in the cultures of interferon-treated transformed cells that had reverted to the non transformed phenotype. It was therefore possible that the induction of reversion of the transformed phenotype was related in some manner to the antiviral action of interferon. It seemed of importance therefore to repeat these studies with another clone of transformed C3H/10T1/2 cells devoid of C-type viral particles and reverse transcriptase activity. The results of the experiments reported herein show that highly purified mouse interferon also induces the reversion of the transformed phenotype of cells of this clone.

Material and methods

Cells. The origin of the X-ray transformed cloned 5T3 cells has been previously described[3]. Cells were cultivated in Eagle's basal medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics in air-5% CO_2 at 37 °C. C-type particles were not detected by electron microscopy and significant levels of reverse transcriptase activity were not demonstrable in these cells using techniques previously described[2].

Interferon. Mouse C-243 cell interferon was prepared and concentrated as previously described[4]. Interferon was purified by affinity chromatography on columns of an anti-mouse interferon antibody and CM-sephadex[5,6]. The specific activity of the interferon was 5×10^8–1×10^9 units/mg of protein. On electrophoresis in polyacrylamide gels in SDS, there were 3 bands corresponding to mol.wts of 30,000, 24,000 and 19,000[6]. Biologic activity was associated only with these bands. Previous work indicates that the interferon bands 30K daltons and 24K daltons correspond to β interferon and the 19K daltons to α interferon (Kawada et al., unpublished results). In the experiments to be reported the highly purified mouse interferon preparation contained both α and β interferons.

Material which was not retained by the anti-interferon antibody column is referred to as 'impurities'. It had a protein concentration of approximately 1 mg/ml and a titer of interferon of about 1:40. After dilution, 'impurities' did not exhibit any antiviral activity.

Analysis of the cell phenotype under prolonged treatment with interferon. Transformed 5T3 cells were passaged once every week in the continuous presence of either 720 units of interferon or impurities (used at the same dilution as interferon). The phenotype of the cells was determined at different passage levels according to criteria of cell proliferation, cell morphology and tumorigenicity as previously described[2].

Electron microscopic examination. Cultures were fixed at room temperature in glutaraldehyde, osmium, dehydrated in ethanol and embedded in Epon as previously described[2].

Table 1. Decrease in tumorigenicity of transformed cells treated with interferon

No. of passages of interferon-treated cultures	No. of cells inoculated/ mouse[a]	No. of mice with tumors/ No. of mice inoculated (range of appearance of the tumors)		
		Control	Impurities	Interferon
1	3×10^6	5/5[b] (2 weeks)	-	5/5 (5–11 weeks)
5	2×10^6	15/15[c] (2–4 weeks)	15/15 (2–4 weeks)	11/11 (4–8 weeks)
18	2×10^6	5/8[d] (6–9 weeks)	-	0/14[e]

[a] Cells were trypsinized, washed once with phosphate saline buffer and suspended in serum-free medium. 3-week-old nude Swiss mice were injected s.c. in the interscapular region with 0.1 ml of the cell suspension. [b,c,d] The number of in vitro passages of transformed 5T3 cells since their isolation was 7, 11 and 24, respectively. [e] The mice were sacrified 24 weeks after inoculation and were autopsied.

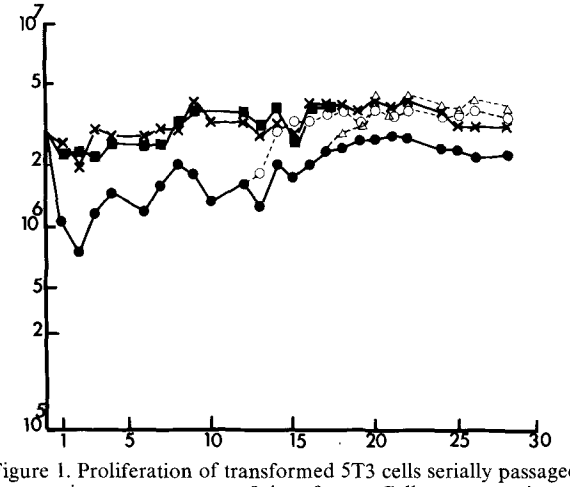

Figure 1. Proliferation of transformed 5T3 cells serially passaged in the continuous presence of interferon. Cell concentration was determined in untreated control cultures (×), in cultures treated with impurities (■), or with 720 units of interferon (●). Cell concentration was also determined after the removal of interferon from the culture at passage 12 (○) and 17 (△). At each passage level the cell concentration was adjusted to 2×10^5 cells/25 cm^2 flask.

Results

Cell proliferation. A clear-cut inhibition of cell multiplication was observed in interferon-treated 5T3 cells compared to the proliferation of untreated cells or cells treated with impurities (fig. 1). This inhibition was pronounced during 13 passages. Thus, at the time of passage, the mean cell density for untreated cultures was $13.04 \pm 1.88 \times 10^4$ cells/cm^2; and $12.64 \pm 2.52 \times 10^4$ cells/cm^2 for cultures treated with impurities, compared with $5.24 \pm 1.24 \times 10^4$ cells/cm^2 for interferon-treated cultures.

At passage 14, a decrease in the extent of inhibition of cell proliferation became apparent. After 20 passages in the presence of interferon, inhibition remained stable. (The mean cell density in interferon-treated cultures was $10.64 \pm 1.52 \times 10^4$ cells/cm^2 compared to $15.16 \pm 1.92 \times 10^4$ cells/cm^2 for untreated cultures).

When interferon was removed from the medium at passages 12 and 17 and cells serially passaged without interferon, they attained cell densities identical to those of untreated cultures after 2–3 passages. (The mean cell density was $13.52 \pm 1.28 \times 10^4$ cells/cm^2 when interferon was removed from the culture at passage 12 and $15.08 \pm 1.04 \times 10^4$ cells/cm^2 when interferon was removed at passage 17).

Cell morphology. Long-term interferon treatment modified the morphology of transformed 5T3 cells (fig. 2). Interferon-treated cells appeared more elongated and aligned in parallel array with no cellular overlapping. This change in cellular morphology was progressive since it occurred between the 6th and 9th cell passages and was maintained thereafter in all subsequent passages.

When interferon was removed from the cultures and the cells serially passaged in the absence of interferon, they gradually regained the transformed morphology 2–3 passages after removal of interferon.

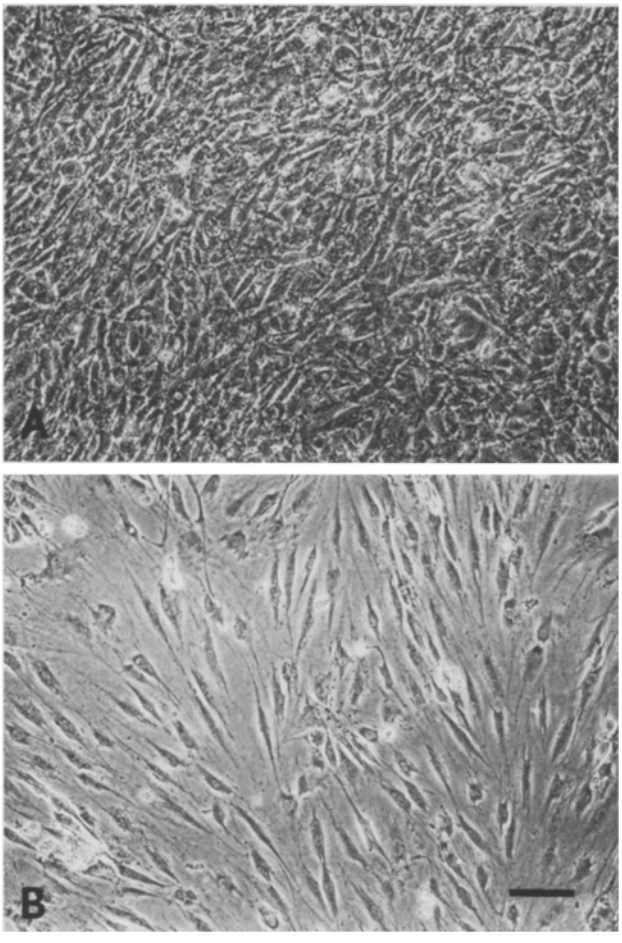

Figure 2. Effect of the treatment with interferon on the morphology of transformed 5T3 cells. *A* Control transformed cells; *B* transformed cells treated for 10 passages with interferon. Bar = 100 µm.

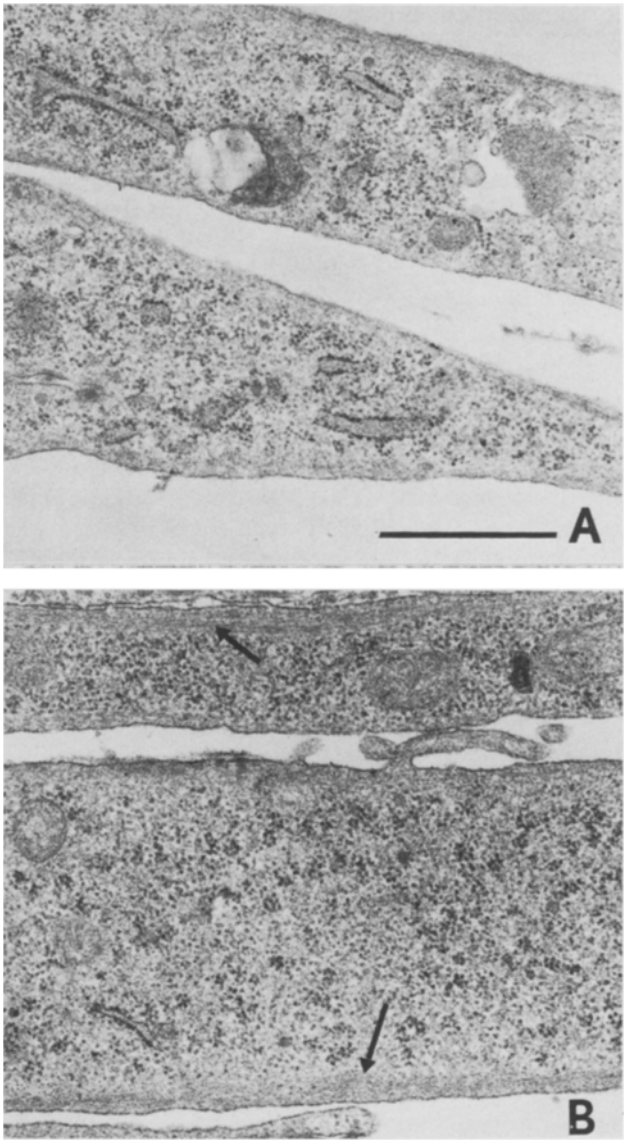

Figure 3. Effect of interferon treatment on the distribution of microfilaments as visualized by electron microscopy. *A* Control transformed cells; *B* transformed cells treated for 18 passages with interferon. Bar = 1 µm.
[Note the thick layer of submembranous microfilaments present in interferon-treated cells and absent in untreated transformed cells.]

Previous studies have shown that changes of the morphologic phenotype induced by interferon were associated with an extensive development of actin-containing microfilaments[7-10]. As illustrated in figure 3, very few cytoskeletal components can be seen in either the cytoplasm or membrane region of untreated transformed 5T3 cells by electron microscopic examination, whereas, abundant microfilaments were observed in the submembranous region of interferon-treated transformed cells.

Tumorigenicity of transformed cells cultivated in the continuous presence of interferon. There was a significant delay in the appearance of the tumors in mice inoculated with transformed cells which had been cultivated for only 1 week in the presence of interferon (table 1). This delay was maintained thereafter in subsequent passages (i.e. passage 5). After 18 passages, in the presence of interferon, none of the mice injected with interferon-treated cells developed tumors (table 1). [At this time, the tumorigenicity of untreated 5T3 cells (which had undergone 32 in vitro passages since their isolation) was less than that of cells from earlier passages (table 1)].

Tumorigenicity of interferon-treated transformed cells after removal of interferon. In our previous studies, we showed that after removal of interferon from interferon-treated cultures, back reversion to the transformed phenotype occurred progressively. First, we observed a back reversion of the morphology and patterns of growth and then subsequently in the tumorigenicity of interferon-treated cells.

As can be seen in table 2 numerous passages in the absence of interferon were necessary for interferon-treated transformed cells to re-express tumorigenicity fully. Interferon-treated cells at passage level 12 required more than 10 passages to recover the tumorigenicity of untreated transformed cells. Similarly, interferon-treated cells at passage 17 were not tumorigenic after 6 passages in the absence of interferon but gave rise to tumors at the 16th passage.

Increasing the number of in vitro passages with interferon did not appear to suppress tumorigenicity since interferon-treated cells at passage 24, gave rise to tumors after 9 subsequent passages in the absence of interferon (table 2).

Table 2. Tumorigenicity of transformed 5T3 cells serially passaged first in the presence of interferon and thereafter in the absence of interferon

No. of passages in the presence of interferon	No. of passages after removal of interferon	No. of mice with tumors/No. of mice inoculated[a] (range of appearance of the tumors)	
		Untreated transformed cells	Interferon-treated cells
12	6	5/8[b] (5-9 weeks)	5/14 (7-17 weeks)
	10	6/10[c] (11-12 weeks)	5/10 (4-20 weeks)
	26	6/6[d] (2-4 weeks)	6/6 (3 weeks)
17	1	5/8[b] (5-9 weeks)	0/14[e]
	6	6/10[c] (11-12 weeks)	0/10[e]
	16	6/6[d] (2-4 weeks)	6/6 (3-5 weeks)
24	9	6/6[d] (2-4 weeks)	6/6 (3-8 weeks)

[a] 2×10^6 cells inoculated s.c. per nude mice. [b,c,d] The number of in vitro passages of transformed 5T3 cells was 24, 29, and 14, respectively. [e] The mice were observed for 6 months.

Discussion

Our results show that highly purified mouse interferon (specific activity 5×10^8-1×10^9 reference untis/mg of protein) induced a progressive reversion of the transformed and malignant phenotype of X-ray-transformed C3H/10T1/2 cells. Thus, interferon-treated transformed cells became aligned without cellular overlapping, grew to low cell density and their tumorigenicity was progressively reduced.

The change in the cell phenotype induced by interferon was not irreversibly fixed, since the characteristic of the transformed phenotype reappeared when the cells were passaged in the absence of interferon (i.e. there was an increased rate of cell proliferation and a morphology characteristic of the transformed phenotype after 2-3 passages, and an increased tumorigenicity after more than 6 passages in the absence of interferon).

The changes in cellular morphology induced by interferon have proven different depending on the cell clones examined (i.e. in the previous[2] and current experiments). For example, both clones of X-ray transformed 10T1/2 cells displayed a fibroblastic-like morphology characteristic of the transformed phenotype before interferon treatment . Cultivation in the presence of interferon led in one instance to the appearance of an epithelioid morphology similar to that of the parental non-transformed 10T1/2 cells[2]. Interferon treatment of these cells was associated with the reappearance of microfilaments throughout the cytoplasm (as was observed by either indirect immunofluorescence[8] or by electron microscopy (unpublished results)). In the experiments reported herein, interferon-treated cells exhibited an elongated morphology similar to that of normal fibroblasts. Here, interferon treatment was associated with the presence of submembraneous microfilaments (fig. 3).

With regard to the tumorigenicity of interferon-treated cells, a significant delay in the appearance of tumors was already evident after 1 passage of transformed 5T3 cells in the presence of interferon (table 1), whereas in our previous work it was necessary to pass cells several times in the presence of interferon before their tumorigenicity was decreased[2].

It seems most likely that these differences in the effect of interferon on the morphology and tumorigenicity can be attributed to inherent differences between the cells of different clones of transformed cells. We stress, however, that interferon-treated cells of both clones fulfilled the criteria for phenotypic reversion. This phenotypic reversion induced in vitro by interferon was obtained in a clone of transformed cells, apparently free of C-type viral particles and reverse transcriptase activity. It seems likely therefore that the effect of interferon in inducing reversion of the transformed phenotype is unrelated to its antiviral activity.

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A computer-based system for collection and analysis of circadian rest-activity data

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Summary. A computer-based system for collection and analysis of circadian rest-activity data was developed. The system has the advantage of a minimal amount of interface hardware and uses standard laboratory computer equipment permitting easy collection and automatic visualization and analysis of the data. The flexibility of the programs allows manipulation of the presentation format as needed and further refinements of the data analyses are possible.

Introduction

Longitudinal recording of motor activity is the principal method that has been used to study rodent circadian rhythms. The rest-activity cycle is usually monitored by means of Esterline-Angus strip chart recordings, the daily plots of activity pasted sequentially below each other, and photographs of double plots used for analysis of period and phase[1]. This method is labor-intensive and does not provide numerical data for quantitative analysis of, for example, the total number of activity wheel revolutions per selected time bin or per 24-h day.

We approached the problem of measuring circadian rest-activity rhythms in animals after several years' experience in applying new electronic and computer technology to the measurement and analysis of human circadian rhythms[2,3]. Since one requirement was to be able to process the animal data in the same way as the human data, it seemed appropriate to apply the more sophisticated data collection and analysis technology to the development of a new computer-based system. Few reports of computer-based rodent motor-activity systems have been found in the literature[4,5]. We present details of a computer-based system that has the advantage of a minimal amount of interface hardware and that uses standard laboratory computer equipment that allows easy implementation in other laboratories. Additionally, we describe the software that was developed to record the circadian rhythm data, and include examples of the computed and plotted results using hamster running-wheel activity data.