a polarization effect can occur (*6*). The sum of these effects over the entire deposit is the observed IP.

It has been brought to our attention (*7*) that there is a need for a method of delineating old dump sites, and we considered the possibility that such sites could be distinguished from their surroundings by the presence of an IP effect due to their disseminated metal content. Accordingly, we made IP surveys over a dump site (*8*) and two separate sanitary landfill sites (*9, 10*).

In all three cases, we observed a definite IP effect. Measured values of metal factor (*MF*) (*11*) were in the range of 50 to 1000. These are to be compared to values of 2 to 30 measured outside the dump site in an adjacent area. In addition, resistivity values correlate with metal factors (Fig. 1). Metal factors observed over mineral deposits range from 10 to over 10,000 (*12*). The histogram of frequency effect values shows a peak around 8 percent (Fig. 2).

Synthetic samples were prepared to study the IP effects in the laboratory. These samples consisted of a mixture of rusty "tin" cans and sand, and new aluminum cans and sand. These gave laboratory values of *MF* from 150 to 350. Frequency effects ranged from 4 to 13 percent. The laboratory value of *MF* for the saturated sand alone is 41 ± 41 with less than 1 percent frequency effect.

It appears that the IP technique may be useful in the investigation of dump sites, if one bears in mind the general problems of interpretation of potential data taken on a two-dimensional plane, that is, the earth surface (*13*). The technique should also be considered for certain archeological sites containing metallic items.

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8. Bedford Town Dump, Bedford, Massachusetts, which was closed in 1969 after being used for more than 25 years as an open-face burning dump. The approximate cross section along the profile consists of 0.3 m of gravel covering about 2½ to 3½ m of dump material. A layer of peat lies beneath the dump material.
9. Hartwell Avenue Sanitary Landfill, Lexington, Massachusetts, which has been used for about 10 years. It has a total depth of about 6 m and consist of alternating layers of dump material and gravelly cover material; each layer is about 15 cm thick.
10. Acton Town Dump, Route 2, Acton, Massachusetts, which was in use between 1915 and 1969 as an open-face burning dump. It has been operated as a sanitary landfill site from January 1970 until present. The cross section consists of about 4½ to 6 m of dump material, overlain by about 0.3 m of gravel.
11. We define the metal factor as $MF = 10^5 FE/\rho_{1.0}$, where the frequency effect $FE = (\rho_{0.1} - \rho_{1.0})/\rho_{1.0}$, resistivities are in ohm-meters, and the subscript indicates the frequency in hertz. The traditional definition of metal factor is $MF = 2\pi \times 10^5 FE/\rho$ with ρ in ohm-feet, which is approximately a factor of 2 larger than our definition.
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Positive Control of Transformed Phenotype in Hybrids between SV40-Transformed and Normal Human Cells

Abstract. *Somatic cell hybrids have been obtained between SV40-transformed Lesch-Nyhan fibroblasts, which are deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and display glucose-6-phosphate dehydrogenase A (G6PD-A) activity, and late-passage HGPRT-positive WI38 human embryo fibroblasts, which display G6PD-B activity. The human-human hybrid clones, which display G6PD-A and G6PD-B and heteropolymers of the two enzyme forms, have the same growth characteristic as the SV40-transformed parental cells and behave as continuous cell lines. The SV40 tumor antigen, the gene for which has been assigned to human chromosome 7, is present in all clones examined.*

The genome of SV40 virus is integrated in the cellular DNA in SV40-transformed cells (*1*), which express several SV40-induced antigens such as tumor (T) antigen, tumor-specific transplantation antigen, and U antigen (*2*). Croce *et al.* (*3*) showed that the SV40 genome and the SV40 T antigen gene are syntenic in SV40-transformed human cells. In addition, the SV40 T antigen gene and the SV40 genome have been assigned to human chromosome 7 in two different SV40-transformed human cell lines (*3*). From the results of Croce *et al.* it also appears that the integration of the SV40 genome in only one chromosome of the chromosome 7 pair is sufficient for expression of the SV40 antigen and for maintenance of the transformed phenotype (*3*). These observations led us to investigate hybrids between SV-40-transformed and normal human cells to determine if the properties of these hybrid cells resemble those of the SV40-transformed parental cells. For this purpose we hybridized SV40-transformed Lesch-Nyhan fibroblasts LN-SV (*3*), which are deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and display glucose-6-phosphate dehydrogenase A

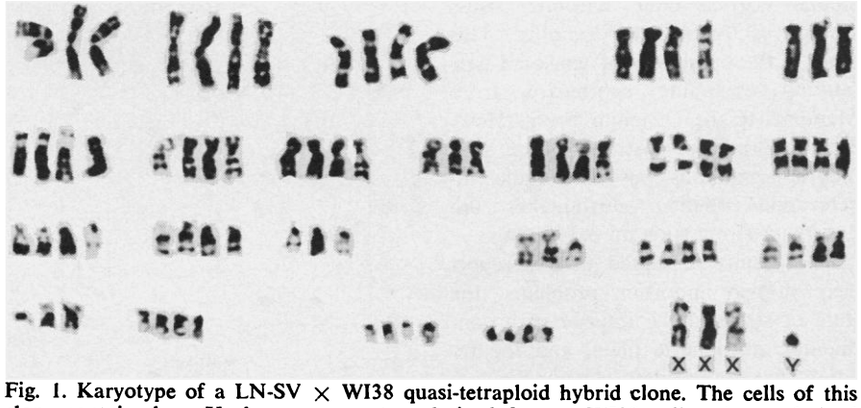

Fig. 1. Karyotype of a LN-SV × WI38 quasi-tetraploid hybrid clone. The cells of this clone contain three X chromosomes, two derived from a WI38 cell and one derived from an LN-SV cell.

(G6PD-A) activity, with the human diploid fibroblasts WI38 (*4*), which are HGPRT-positive and display G6PD-B activity.

Seeding of WI38 cells (at passage 37) at the low density of 100 cells per 60-mm petri dish did not result in the formation of any visible cell colony within 14 days after seeding. The LN-SV cells were positive for SV40 T antigen, and their average chromosome number was 56. Approximately 75 percent of the LN-SV cells were diploid; a minority were quasi-tetraploid (average chromosome number of 86). When 100 LN-SV cells were seeded per 60-mm petri dish, 35 LN-SV colonies per dish appeared within 14 days.

The parental cells were fused with β-propiolactone–inactivated Sendai virus in Eagle's minimal essential medium at *p*H 8.0 (*5*), and the hybrid cells were selected in hypoxanthine-aminopterin-thymidine (HAT) medium (*6*) as described (*7*). Colonies of hybrid cells were picked and then grown continuously in HAT medium. Cells derived from each colony were cloned in microtest plates, and 15 clones, each derived from a different colony, were analyzed. Some of the clones originally selected in HAT medium were back-selected in medium containing 8-azaguanine (30 μg/ml) to obtain human-human hybrids that have lost the active X chromosome of the WI38 cell. Approximately one colony of cells growing in 8-azaguanine medium per 10^5 cells seeded was obtained from back-selection. The resulting hybrid cells selected in HAT medium displayed human HGPRT activity and human G6PD-A and G6PD-B and heteropolymers of the two enzyme forms. Clones back-selected in 8-azaguanine displayed only G6PD-A activity (Table 1).

All clones examined (Table 1) were positive for SV40 T antigen in an indirect immunofluorescence test; 100 percent of the cells of each of the 15 hybrid clones tested were positive for SV40 T antigen. This indicates that the human chromosome 7 carrying the SV40 T antigen gene (*3*) and the SV-40 genome (*3*) is very rarely lost, if ever. All of the hybrid cell clones behaved as continuous cell lines; they have been transferred for more than 45 passages since their isolation. The chromosome number of the hybrid clones was close to that expected for this type of hybrid (Table 1) (*8*). Karyological analysis after Giemsa banding staining (*9*) (at least 25 metaphases examined for each clone studied) indicated that all of the hybrid cells were very close to tetraploidy (Fig. 1). No consistent pattern of reduction or increase in the number of chromosomes of any specific human chromosome pair was observed in all 15 HAT clones examined.

Table 1. Expression of the transformed phenotype in hybrids between normal and SV40-transformed human cells. Abbreviations: CI, contact inhibition; T, SV40 T antigen; AB, heteropolymer of G6PD-A and G6PD-B.

Cells	CI	T	G6PD	Average chromosome number
LN-SV	−	+	A	56
WI38	+	−	B	46
Hybrid clone:				
1	−	+	A + B + AB	96
2	−	+	A + B + AB	87
4	−	+	A + B + AB	87
7	−	+	A + B + AB	85
11	−	+	A + B + AB	89
17	−	+	A + B + AB	93
21	−	+	A + B + AB	87
22	−	+	A + B + AB	83
25	−	+	A + B + AB	89
28	−	+	A + B + AB	87
30	−	+	A + B + AB	85
35	−	+	A + B + AB	91
41	−	+	A + B + AB	93
49	−	+	A + B + AB	88
67	−	+	A + B + AB	90
1 8-aza*	−	+	A	90
21 8-aza*	−	+	A	91

* Clone back-selected in medium containing 8-azaguanine (30 μg/ml).

All of the 17 clones examined had a plating efficiency close to that of the SV40-transformed parental cells (27 to 39 colonies per 100 hybrid cells seeded per 60-mm petri dish). The morphology of the cells of all clones examined was similar, if not identical, to that of SV40-transformed cells, and all had the same mitotic index. In addition, none of the human-human clones tested displayed the properties of density-dependent inhibition of cell division which are characteristic of normal cells (Table 1), and all tended to pile up in culture.

It has been postulated that the balance between "expressor" (E) and "suppressor" (S) chromosomes is the determinant of virally or chemically induced transformed cells in hamsters (*10*). These authors suggest that if E > S the cells are malignant and if S > E the cells are not malignant. If S > E cells induce tumors in hamsters, these authors suggest that some of the cells have become E > S and "reverted" to the tumorigenic state. The phenotype of hybrids between normal diploid fibroblasts, which never become spontaneously transformed, and LN-SV cells is clearly transformed, and the hybrid cells cannot be distinguished from the transformed parental cells on the basis of growth properties. Since these hybrid cells are quasi-tetraploid (Table 1 and Fig. 1) and no consistent pattern of reduction or increase in the number of chromosomes of any specific human chromosome pair was observed in the hybrids, it seems unlikely that an S > E chromosome balance results in the suppression of the transformed phenotype.

It has also been postulated that hybrids between tumorigenic and normal cells are generally normal unless the chromosomes of the normal cells are lost from the hybrids (*11*). This postulate also seems unlikely in the case of hybrids between SV40-transformed and normal human cells. In our study, all of the hybrid cell clones were quasi-tetraploid, were clearly transformed, and expressed the SV40 T antigen. Apparently, the integration of the SV-40 genome in one or two of the human chromosomes of pair 7 of one of the parental cells is sufficient for the maintenance of the transformed phenotype in the hybrids with normal human diploid fibroblasts.

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