Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 570–574, January 1985
Medical Sciences

Identification of a single chromosome in the normal human genome essential for suppression of hamster cell transformation

(anchorage independence/baby hamster kidney cells/human chromosome 1)

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Communicated by David Shemin, September 24, 1984

ABSTRACT **Normal human fibroblasts were fused to carcinogen-transformed baby hamster kidney (BHK) cells and found to be able to suppress the anchorage-independent transformed phenotype of the hamster cells. This suppression was not due to interspecies incompatibility, for transformation could be effectively expressed in hybrids if either the human or the BHK parent had initially been transformed by a dominantly acting viral genome. Upon growth of suppressed hybrids, loss of human chromosomes was accompanied by the re-expression of transformation. Karyotype analysis indicated that only human chromosome 1 was retained in all hybrids that were suppressed and was lost in all hybrids in which transformation was re-expressed. Cytological evidence for the presence or absence of chromosome 1 was confirmed by electrophoretic identification of the human isozyme for phosphoglucomutase 1. Clones re-expressing transformation were isolated from two suppressed hybrids and in both cases loss of suppression was accompanied by the loss of human chromosome 1. Thus, the maintenance of suppression in these cross-species hybrids appears to require the continued presence of normal human chromosome 1. These findings raise the possibility that the frequent involvement of human chromosome 1 in potentially inactivating aberrations in human tumors may reflect a suppressor role for this chromosome in human malignancy.**

In most naturally occurring human malignancies, tumorigenicity behaves as a recessive trait. Several lines of evidence associate the inactivation of a diploid pair of suppressor alleles with the development of retinoblastoma (1, 2), and evidence is accumulating that similar events are involved in the genesis of other hereditary tumors (3–5). *In vitro* cell fusions both within and across species involving human tumor lines not derived from hereditary cancers also indicate that malignancy is recessive (6–12). This recessiveness implies that during the process of carcinogenesis there has been a loss of some function that maintains normal constraints on growth. As suggested first in 1973 by Comings (13), such recessive loci whose function is apparently lost during tumor development could be structural genes whose products act directly to prevent autonomous growth in normal cells or they could be regulatory genes that act by suppressing the function of second genes, possibly oncogenes, coding for transforming factors.

Except for hereditary tumors, which could be special cases, tangible evidence for the existence of such descrete suppressor loci in normal human cells has been lacking. In the mouse, however, chromosomes marked with natural polymorphisms have been used to map to mouse chromosome 4 a locus able to suppress the malignancy of four different mouse tumors (14). In the human genome, chromosomes 11 and 14 have been suggested as suppressors of tumorigenicity in fibroblast–HeLa cell hybrids as two tumors derived from suppressed hybrids have lost one copy of each of these autosomes (15). Unfortunately, in these intraspecies hybrids it is not possible to be sure that it is the homologue derived from the normal parent that has been lost. Extensive hybridizations between malignant rodent and normal human cells, in which chromosomes of each parent are easily distinguished, have shown that suppression, when it is possible to analyze it, is associated with a constellation of chromosome pairs. No single human chromosome has been identified as able to maintain suppression alone or even as being crucial to its maintenance (8–10, 16).

The apparently complex array of possible human suppressor chromosomes identified to date may reflect the multistep nature of carcinogenesis as well as the multiplicity of cultured cell characteristics that can enhance *in vivo* tumorigenicity. Therefore, we have sought to define and map in the human genome a function that is responsible for the suppression of a simple, single step in carcinogenesis—namely, the expression of anchorage independence in hamster cells. We have used a line of pseudodiploid Syrian hamster fibroblasts, BHK 21/13 (17). In this immortal cell line, anchorage independence can be shown to arise in a single mutagenic step (18, 19) and to be directly correlated with what is for these cells the last of the progressive changes leading to tumorigenicity. The anchorage independence of transformed BHK cells is suppressed by fusion with the anchorage-dependent, normal parent line and such suppressed hybrids display reduced tumorigenicity (unpublished data).

In this paper we report that anchorage independence is also effectively suppressed by fusion of transformed BHK cells to normal human fibroblasts, that this suppression is not due to cross-species incompatibility, and that the continued presence of human chromosome 1 is required to maintain suppression.

EXPERIMENTAL PROCEDURES

Cells and Culture Conditions. Hamster cells, all derivatives of BHK 21/13 (17), were carried routinely in Dulbecco modified Eagle's medium (DME medium) with 10% donor calf serum and 10% tryptone phosphate broth (TP broth). Hybrids and transformants were maintained at 38.5°C and other cells were maintained at 36.5°C. SN-10 is a normal subclone of BHK 21/13 (18), and DMN 4A (20) is a carcinogen-induced, anchorage-independent transformant. Their ouabain-resistant and 6-thioguanine-resistant derivatives have been described (19). SV-28, a BHK clone transformed by simian virus 40 (SV40), was obtained from A. Smith and was made resistant to 3 mM ouabain and to 100 μM 6-thioguanine.

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Abbreviations: BHK, baby hamster kidney; HAT, hypoxanthine/aminopterin/thymidine; SV40, simian virus 40; PGM_1, phosphoglucomutase 1.

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Human cells were cultured in DME medium with 10% fetal calf serum. Normal human embryo strain A8097, from the Naval Biosciences Laboratory (Oakland, CA), was used at passages 4–13. SK-1b and 25-SK are strains derived from normal human foreskins and were used at passages 4–10. Growth slows and senesence begins for these lines after 20 passages. A293, a human line transformed by the adenovirus early region E1 (21), was supplied by B. Thimmappaya.

Fusions. Twenty-four hours after being plated at parental ratios of between 1:1 to 1:10 (the human parent in excess), cells growing in monolayer were fused as described (19) by a 60-sec exposure to 50% polyethylene glycol 1000 (PEG 1000) prepared in 2× concentrated DME medium. In some experiments, calcium-free DME medium was used for washing out the PEG. Fused cells were incubated overnight at 38.5°C, harvested, and assayed for total hybrids by plating at 2–10 × 10^4 cells per 100-mm dish in hypoxanthine/aminopterin/thymidine (HAT)/ouabain medium containing DME medium with 10% fetal calf serum, 10% donor calf serum, 4% TP broth, 1 μM ouabain, 0.1 mM hypoxanthine, 0.5–5 μM aminopterin, and 16 μM thymidine. A portion of cells was assayed simultaneously for anchorage-independent hybrids by plating 2 × 10^5 cells per 60-mm dish in HAT/ouabain medium made with 5% of each serum and solidified with 0.3% agar (22). Liquid platings were refed weekly and counted at 2–3 wk, and agars were counted at 3–4 wk. All cells were checked at the time of fusion by Hoechst stain for mycoplasma infection and found to be negative (19). Control fusions of each parent to itself were included in each experiment and were consistently negative.

Hybrid Analysis. Progressively growing hybrid clones were isolated from HAT/ouabain liquid plates. Except where designated, care was taken to derive only one hybrid from each initial fusion to avoid isolating sister clones. After recloning in selective media, hybrids were assayed for the expression of transformation in selective media by plating at 1–20 × 10^3 cells in soft agar and for cloning efficiency by plating at 200 cells per 60-mm dish in liquid media. The transformed phenotype was considered to be expressed if the relative agar plating efficiency of a hybrid clone (the percent of cells able to clone on plastic that are also able to clone in soft agar) was ≥3.7% and to be suppressed if the relative agar plating efficiency was ≤0.4%. A portion of the same hybrid cell suspension assayed for expression of transformation was also plated for chromosome analysis. Within 3 days, cells were arrested with 0.01 μg of colcemid per ml and trypsin/Giemsa-banded chromosome preparations were prepared by standard methods (23). Ten to 18 metaphases were analyzed per hybrid. BHK chromosomes were identified and segregated and human chromosomes were identified. A chromosome was considered to be present in the hybrid if 10% or more of the cells contained one or more copies. Chromosomes present only in part were not included in the analysis.

Hybrids were tested for biochemical evidence of the presence of human chromosome 1 by detection of human phosphoglucomutase 1 (PGM_1, EC 2.7.5.1). Lysates were prepared by freeze-thawing cells three times and were electrophoresed on cellulose acetate strips 7.5 cm wide (Helena Laboratories, Beaumont, TX) using buffers and stain recommended by van Someren *et al.* (24). Samples were applied 2.5 cm from the cathode and run for 2 hr at 4°C at constant amperage with initial voltage set at 200 V across a gap of 8.5 cm. A portion of the hybrid cells used to prepare each lysate was checked for expression of transformation by plating in liquid and in agar as described above.

RESULTS

The anchorage independence of carcinogen-transformed BHK cells is effectively suppressed by fusion to a normal BHK cell line (19). This suppression is temporary, for the original transformed phenotype is re-expressed after extensive growth of the hybrid. To determine if normal human fibroblasts can substitute for the normal BHK cells in this suppression, three different strains of normal human cells—two derived from foreskin and one from an embryo—were fused to a chemically transformed BHK clone that was ouabain and 6-thioguanine resistant. Twenty-four hours after fusion the cell mixture was suspended and one portion was assayed for total hybrids by cloning on plastic in HAT/ouabain medium and a second portion was assayed for hybrids expressing transformation by plating in HAT/ouabain medium containing 0.3% agar. Table 1 summarizes a series of such experiments. BHK cell transformation as measured by anchorage independence is suppressed by fusion to a normal human fibroblast, just as it is upon fusion to a normal BHK cell. When the same parent is fused to a carcinogen-transformed BHK cell, transformation is not suppressed.

Assay of hybrids at such early times after fusion minimizes the masking of an initial phenotype by rapid or nonrandom chromosome loss, which can be a problem in unstable interspecies hybrids, and also allows the screening of many more hybrids than possible if the individual clones must be picked and tested. Experiments with normal–transformed BHK cell fusions have shown that there is no significant change in hybrid phenotype when hybrids assayed at 48 and 72 hr are compared to those assayed at 24 hr.

Individual hybrids arising on plastic without overt selection for or against transformation were also picked, recloned, and assayed for the suppression or expression of transformation. Approximately equal numbers of hybrids suppressing and expressing transformation were obtained (Table 2), indicating that as the hybrids grow and preferentially lose human chromosomes, initial suppression is frequently lost.

To control against the possibility that the BHK–human hybrids, although able to grow on plastic, are unable to grow in agar soon after fusion for some trivial reason unrelated to transformation, similar cross-species fusions were performed by using dominantly transformed cells to provide a positive control. Although spontaneously arising and chemically induced transformation is usually a recessive trait, transformation induced by DNA viruses is dominant in many cell lines, including BHK (19). When human cells transformed by adenovirus region E1 were fused to transformed BHK cells or when SV40-transformed BHK cells were fused to normal human fibroblasts, transformation was efficiently expressed at 24 hr (Table 1).

Since chemically induced BHK cell transformation occurs in a single step and temperature-sensitive transformants are common, it is thought to be the result of a single lesion (18, 19). It would be expected that suppression by hybridization to a normal cell would depend on a single factor, which should map to a single location in the normal cell genome. To map the human cell function responsible for the suppression of BHK cell transformation, 11 hybrids were isolated following the fusion of chemically transformed BHK cell clone DMN 4A, which was ouabain and 6-thioguanine resistant with human foreskin fibroblasts (Table 2). Strain SK-1b was the human parent in all fusions except those leading to hybrid 1-107 B-A, where 25-SK was used, and to 20-2gAg, where A8097 was used. All hybrids derive from independent fusions with the exception of 1-82 C-b and 1-82 B-Aag, which originated as separate primary clones in the same fusion experiment. Following recloning, hybrids were assayed simultaneously for the expression of transformation, as measured by relative plating efficiency in soft agar and plated for preparation of trypsin-banded chromosomes. Agar assays indicated that about half of the recloned hybrids were re-expressing transformation (Table 2). Chromosome analysis

Table 1. Expression of transformation in hamster–human hybrids tested 24 hr after fusion

Fusion	Relative parental agar plating efficiency, %*	Hybrids, no. per 10^6 cells tested	% hybrids expressing transformation	Suppression of transformed phenotype
Carcinogen-transformed BHK†	23			
With carcinogen-transformed BHK (DMN 4A)	95	250	53	−
With normal BHK (SN-10)	0.034	783	0.9	+
With normal human fibroblasts‡				
A8097	<0.0004	675	<0.7	+
SK-1b	<0.0007	110	<2.3	+
25-SK	<0.0002	140	<1.7	+
Normal human fibroblast (SK-1b)	<0.0007			
With carcinogen-transformed BHK†		138	0.09	+
With SV40-transformed BHK§	43	363	108	−
Adenovirus-transformed human (A293)	19			
With normal BHK (SN-10)	0.028	1140	47	−
With carcinogen-transformed BHK†		1430	65	−

*Calculated as (proportion of cells cloning in soft agar/proportion of cells cloning on plastic) × 100.
†DMN 4A ouabain and 6-thioguanine resistant 1bR.
‡Average of 3 experiments for A8097 and 10 experiments for SK-1b.
§SV-28 ouabain and 6-thioguanine resistant 3F.

showed all hybrids contained variable numbers of human chromosomes, always less than a 2*n* complement, and a subtetraploid number of BHK chromosomes, as has been observed in other human–hamster hybrids (10, 16).

The specific human chromosome content of each primary hybrid is listed in Table 2. All human chromosomes show multiple discordancies with the suppression of transformation, except chromosome 13, which shows only one, and chromosome 1, which shows none. Two of the suppressed hybrids containing chromosomes 1 and 13 were plated at high density in agar and rare clones were picked that were re-expressing transformation. Karyotypes of these subclones (1-68 C-CAg and 1-65 bc-AAg, Table 2) showed that in both clones chromosome 13 was retained. Chromosome 1 was lost completely in one of these hybrids. In a second, the normal chromosome 1 observed in the suppressed parent was lost from all metaphases, but in 8 of the 17 metaphases examined a marker chromosome was present, a portion of which was similar to the p arm of chromosome 1 (Fig. 1). This marker chromosome could also be seen along with a normal-appearing chromosome 1 in metaphases of the suppressed hybrid parental clone.

When all clones examined are tabulated and the presence or absence of each chromosome compared to the presence or absence of the suppression of anchorage independence, only chromosome 1 shows no discordancies (Table 2). For all other chromosomes there are 3 or more discordant clones. The presence of chromosome 1 thus seems to be associated with the suppression of BHK cell transformation.

Only one copy of chromosome 1 appears sufficient for suppression for in only 2 of the 5 suppressed hybrids was chromosome 1 present in more than one copy per cell, and in only 22% of the total metaphases from these 2 hybrids were two copies seen.

Table 2. Correlations between the presence of human chromosomes and the suppression of anchorage independence in isolated hybrids

Hybrid	Relative agar plating efficiency, %	Suppression of transformation	Human chromosome* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	Human PGM_1†
Primary																											
1-107 B-A	<0.04	+	0.28	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)
1-106 D-A	8.9	−	−	−	−	−	−	−	+	−	−	−	+	+	−	+	−	−	−	+	−	+	−	−	+	+	−
1-102 G-Z	13	−	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
1-101 I-D	<0.04	+	0.45	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1-91 C-A	<0.09	+	0.20	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	ND
1-82 B-Aag	56	−	−	−	+	−	−	−	+	−	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	−	−
1-68 C-C	0.4	+	0.36	+	−	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
1-65 bc-A	0.22	+	0.36	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
1-99 A-A	3.7	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	+	−	−
20-2g-Ag	30	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	−	−
1-82 C-b‡	<0.09	+	0.18	−	+	−	−	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	ND
Re-expressing																											
1-68 C-CAg§	14	−	P	+	−	−	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+
1-65 bc-AAg¶	5.8	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−
Total discordant clones			0	4	6	3	4	3	6	5	3	4	6	5	3	6	3	5	3	5	4	6	4	5	7	3	

*Presence of a chromosome is indicated by +; absence is indicated by −. P indicates the presence only in part. Actual frequency is specified for chromosome 1.
†Presence of PGM_1 with mobility corresponding to that of the human parent is indicated by +; absence is indicated by −. ND, not done; (+), light human band.
‡Isolated from same fusion as hybrid 1-82 B-Aag.
§Selected as a clone growing in soft agar from hybrid 1-68 C-C.
¶Selected as a clone growing in soft agar from hybrid 1-65 bc-A.

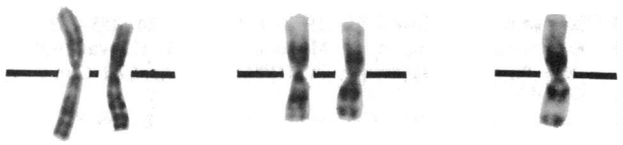

FIG. 1. Marker chromosome containing part of human chromosome 1. Identifiable whole and partial human chromosome 1s are shown from metaphases of the normal human fibroblast strain SK-1b, which has two normal chromosome 1s (*A*), of the suppressed hybrid 1-68 C-C, which has one normal chromosome 1 and one marker (*B*) and of its derivative 1-68 C-CAg, which is re-expressing transformation and which has no normal chromosome 1 and one marker (*C*).

In all suppressed hybrids in this series, chromosomes 7, 11–15, 17–21, and X are always present along with chromosome 1. However, each of these chromosomes can be retained when suppression is lost. In one hybrid, 1-102 G-Z, every one of these chromosomes was present without chromosome 1 and yet the hybrid was not suppressed. Neither together nor separately does this set of chromosomes seem to suppress transformation in the absence of chromosome 1. No significant increase in the copy number per cell of these chromosomes could be detected in suppressed hybrids compared to the nonsuppressed hybrids.

Because of the association of chromosome 1 with suppression, cytological evidence for its presence or absence was confirmed in 11 of the 13 hybrid clones by electrophoretic identification of PGM_1 isozymes. PGM_1 has been mapped to band p22.1 of human chromosome 1. Bands corresponding to both human and BHK forms of PGM_1 were seen in suppressed hybrids in which a cytological chromosome 1 was seen and the human bands were absent from nonsuppressed hybrids in which it was not seen, with one exception (Table 2). The re-expressing hybrid clone 1-68 C-CAg showed a human PGM_1 band. This could be explained by the cytological observation noted above that in almost half of the metaphases analyzed for this clone, the part of the p arm of chromosome 1 on which PGM_1 is located was present in a marker chromosome.

DISCUSSION

Analysis of a series of hybrids formed between a carcinogen-transformed clone of BHK cells and normal human fibroblasts shows that the suppression of the anchorage independence in these hamster fibroblasts requires the continued presence of human chromosome 1. In addition, anchorage-independent clones selected from suppressed hybrids in both cases tested have lost human chromosome 1. Although chromosome 1 is the only human chromosome that is absolutely required for suppression, data are not yet extensive enough to determine whether it performs this function alone or in concert with any one of 12 other chromosomes that are consistently present along with chromosome 1 in all suppressed hybrids. The data do show clearly, however, that these other chromosomes, either individually or as a group, are not able to suppress in the absence of chromosome 1.

Human chromosome 1 is not among those chromosomes that are associated in various combinations with the suppression of malignancy of either Chinese hamster CHO (16) or DON (10) cells or of human HeLa cells (15). This discrepancy could arise in part from the fact that the two lines on which most extensive work has been done are of epithelial origin, whereas BHK cells are fibroblasts. Prior studies have usually identified more than one chromosome that must be lost to insure the re-expression of malignancy (10, 15, 16). This may reflect the complexity of the nude mouse tumorigenicity assay used compared to the simplicity of the anchorage-independent phenotype for which we find loss of only chromosome 1 is sufficient for re-expression. In the case of CHO cells, the range of different chromosome pairs implicated as suppressors of malignancy (16) can be explained if it is assumed that the borderline tumorigenicity of this line depends on the sum of a large number of cellular characteristics. Suppression of any small number of these traits could abrogate the tumorigenic response of the line.

Two studies of mouse hybrids have indicated that a single copy of any chromosome from the normal parent, whether mouse or human, can be present in tumor cells derived from hybrids between normal and malignant cells and thus suggest that no single chromosome can act alone as a suppressor of tumorigenicity (8, 25). However, it has been argued by Harris (14) that dosage effects are crucial to the expression of tumorigenicity in the mouse lines used in these experiments and thus that suppression can only be expected if the number of suppressor chromosomes equals or exceeds the number of transformed cell chromosomes being suppressed. One suppressor chromosome may thus be insufficient. It is difficult to assess dosage effects on the suppression of transformation observed in the experiments reported here, for, although one copy of human chromosome 1 appears sufficient, all hybrids are subtetraploid for BHK chromosomes, BHK cells themselves are functionally hemizygous at the locus involved in transformation (18, 19), and the location and therefore the dosage of transforming BHK lesions in the hybrid cells are unknown.

The idea of suppressor chromosomes active in the human genome at first seems at odds with the concept of dominant-acting oncogenes (27), but 80% of human tumor DNAs are not positive in assays for such oncogenes (26, 27) and it is not yet clear whether such genes are indeed dominant *in situ*, where at least their proto-oncogenes are subject to regulation during development (28) and regeneration (29) and in cell hybrids (30). In several human tumor lines and in two human tumor DNAs that contain a mutationally activated K-*ras* oncogene, the normal allele is absent, as would be expected for a recessive gene (31). Recently, two suppressed hybrids have been isolated from a fusion of HT1080 with normal human fibroblast, and, although an N-*ras* gene dominantly active on NIH/3T3 cells can be isolated from this line (32), malignancy is suppressed in the hybrids (33).

Although the data presented in this paper define human chromosome 1 only as a suppressor of BHK cell transformation, several pieces of evidence indicate that it might also act to suppress malignancy *in vivo*. First, human chromosome 1 shares a large region of homology with mouse chromosome 4. Six of the eight biochemical markers on mouse chromosome 4 are present on the short arm of human chromosome 1 (34), and normal mouse chromosome 4 is capable of suppressing the malignancy of several mouse tumor lines (14). Second, anchorage-independent transformation of BHK cells is highly correlated with neoplasia. Normal BHK cells are not tumorigenic when injected into young hamsters at doses that exclude from the inoculum any spontaneously arising, anchorage-independent transformants (ref. 35; unpublished observations). Anchorage-independent BHK transformants are tumorigenic in 100% of animals at doses as low as 10 cells per animal if protected with 10^4 normal cells. In addition, populations of suppressed hybrid cells exhibit a much decreased tumorigenicity and have an extended latent period that is identical to that resulting from the injection of reconstructed mixtures of normal and transformed cells that have been arranged to reflect the ratio of anchorage-dependent and -independent cells that are present in the hybrid population (unpublished data). Marshall and Dave have also observed anchorage independence and tumorigenicity of a

BHK cell line to be suppressed together in intraspecific hybrids (36).

Pseudodiploid Syrian hamster BHK cells are distinctly different from the CHEF line that is derived from the Chinese hamster and employed by Sager and co-workers to explore the genetics of malignancy (37). Although in fusions CHEF cells show initial suppression of both anchorage independence and tumorigenicity (37), in newly arising transformants anchorage independence does not necessarily correlate with tumorigenicity (38, 39). Diploid CHEF cells apparently must undergo at least three separate changes in order to become malignant, and anchorage independence, representing only one of these changes, is not by itself sufficient for tumorigenicity. CHEF cell anchorage independence resembles the premalignant morphological transformation seen in Syrian hamster embryo cells, which also is inducible at frequencies that are orders of magnitude greater than induced mutation frequencies, and is also by itself insufficient for the expression of tumorigenicity (40). The BHK cell line, on the other hand, behaves as if all of the various progressive changes required for tumorigenicity have already occurred except one—anchorage independence. When anchorage independence is induced in BHK, it occurs in a single, usually mutagenic, step at a single locus at a frequency similar to the frequency of other mutations, and simultaneously the cells appear to become malignant (refs. 19 and 20; unpublished data).

The possibility that human chromosome 1 may also have a suppressor effect on the malignancy of human cells is raised by the very high frequency with which chromosome 1 abnormalities are found in human tumor karyotypes (41–45). Although some of these changes may be random or more relevant to proliferation of tumors once established (46–48), others seem directly relevant to malignancy (49), and many, like those breaks, deletions, and rearrangements that are associated with some melanomas and meningiomas (42) and with the majority of neuroblastomas (50), are of a configuration that can easily be interpreted as leading to loss of function of suppressor loci on chromosome 1. More direct evidence for an involvement of chromosome 1 in human malignancy comes from a recent analysis of three tumors derived from a suppressed hybrid originally produced by fusion of the human fibrosarcoma line HT1080 with a normal human fibroblast. Coincidentally with regaining the ability to form tumors in nude mice, this hybrid also lost two copies of chromosome 1 and one copy of chromosome 4 (33). More direct tests using marked chromosomes in human–human hybrids should reveal whether human chromosome 1 functions to suppress malignancy of human cells *in vivo* as it suppresses hamster cell transformation *in vitro*.

We wish to express our gratitude to W. Fahl, A. Smith, and B. Thimmappaya for generously sharing cell strains and lines, to A. Martin and to J. Beaird for invaluable advice on chromosome identifications, and to P. Iannaccone for crucial help with cellulose acetate gels and critical reading of the manuscript. This investigation was supported by Public Health Service Grant CA 27306 awarded by the National Cancer Institute.

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