Transformation of Bloom's Syndrome Fibroblasts by DNA Transfection

Abstract. *Nonmalignant diploid human fibroblast cells (GM3498B) derived from a skin biopsy of a patient with Bloom's syndrome have been transformed by transfection with DNA from a tumorigenic mouse cell line (Ha-8) carrying a single copy of the Harvey murine sarcoma virus (Ha-MuSV) genome. The transformed cell lines have an extended life-span, form colonies in agarose, and proliferate in nude mice—characteristics of neoplastic transformation. Like the parental cells, they also exhibit a high spontaneous level of sister chromatid exchanges. Finally, the transformed cells contain most, if not all, of the Ha-MuSV genome as well as the human* ras^H *sequence. These experiments show that these diploid nonmalignant human cells can be used as recipients in transfection experiments for studying the genetic control of neoplastic transformation.*

Transfection of DNA isolated from neoplastic cells into nonneoplastic recipient cells is an effective approach for the study of the genetic control of cancer. Tumorigenic cells from a variety of mammalian organisms contain DNA-transforming sequences that control the expression of the neoplastic state (*1–10*). These tumorigenic cells have been derived from cells transformed in vitro by chemicals or viruses and from both spontaneous and induced tumors. DNA-transforming sequences identified in human tumors fall into two classes, one with and one without sequences homologous to retroviral oncogenes. These human retroviral-related DNA-transforming sequences all contain homologs of the *ras* family (*9–12*). The DNA-transforming sequence from a bladder carcinoma cell line has been isolated in several laboratories (*8, 13, 14*); sequence analysis indicates that the transforming activity is the result of a single base change in the ras^H gene (*15–17*). Thus, transfection studies provide relevant information for determining the molecular basis for the control of the tumorigenic phenotype.

NIH/3T3 (3T3) cells have been commonly used as recipients for identification and isolation of DNA-transforming sequences (*1–10*). Similiar experiments with human cells would be useful for studying the regulatory effects of human DNA-transforming sequences on growth and development of nonmalignant human cells and for the isolation and identification of DNA-transforming sequences from other species. Experiments with human cells may resolve questions concerning the relevance of data obtained when rodent cells are used as receptors. Our study demonstrates that human fibroblast cells can be transformed by DNA transfection. The recipient cells, GM3498B, were isolated from a patient with Bloom's syndrome, an autosomal recessive disease characterized by growth retardation, sun sensitivity, immunodeficiency, and an increased predisposition to an early onset of cancer (*18*). The cells, derived from a skin biopsy of nonmalignant tissue, have a finite life-span in vitro and do not express any marker of transformation.

DNA from Ha-8, MRC-5, or 3T3 cells, or from salmon sperm (75 μg of each in five dishes) were used to transfect GM3498B cells. Only cultures treated with Ha-8 DNA developed foci with an altered cellular orientation characterized by a piling up of rounded and hyperrefractile cells at the center and crisscrossed pattern of spindle-shaped fibroblasts at the periphery (Fig. 1A). The Ha-8 cell line [designated Ha-Balb in (*19*)] was derived from NIH/3T3 mouse cells transformed by Harvey murine sarcoma virus (Ha-MuSV) DNA. These cells have a single copy of Ha-MuSV DNA incorporated in their genome (*20*) and do not produce viral particles. In contrast, no focus was present in cultures treated with DNA from nontransformed MRC-5, 3T3, or salmon sperm cells, or with calcium phosphate transfection buffer alone. In these dishes, the confluent cultures maintained an oriented cell pattern during 5 weeks of observation after DNA treatment. The observed frequency of transfection with Ha-8 DNA was 0.16 foci per microgram of DNA, consistent with that observed with NIH/3T3 cells as recipients (0.1 to 0.5 foci per microgram) (*21*).

Progressively growing cultures were obtained from 2 of 12 foci; the rest failed to grow beyond four passages (Table 1). The two progressively growing cell lines, designated GM3498B-HV1 and GM3498B-HV2, were subcultured weekly at a ratio of 1:4; these cultures have an indefinite life-span, whereas the GM3498B parental culture ceased to grow after 19 passages (five passages after the initiation of transfection).

Anchorage-independent growth is often considered a marker of malignancy because it correlates with the tumorigenicity of animal cells transformed by various carcinogenic agents (*22–24*). Growth in agarose after 3 weeks of incubation was observed in three separate experiments only with GM3498B-HV1 and GM3498B-HV2 cell lines (Fig. 1B and Table 1). The two cell lines had a similar frequency of colony formation and a similar colony size. The colonies, with approximately 2000 cells after 4 weeks of growth, lacked a well-defined circumference.

Nude mice inoculated intracranially with GM3498B-HV1 or GM3498B-HV2 cells developed neurological dysfunction 6 to 8 weeks after injection (Table 1). Those inoculated with parental GM3498B cells showed no such effects. Some animals were killed when locomotor difficulties occurred; histological brain preparations showed fibroblastic cellular infiltration of the cerebral hemisphere, evidence of neoplastic potential. The neurological dysfunction resulted in progressive paralysis and death within 10 days of onset. Mice injected with GM3498B parental cells lived at least 6 months without developing abnormal neurological symptoms.

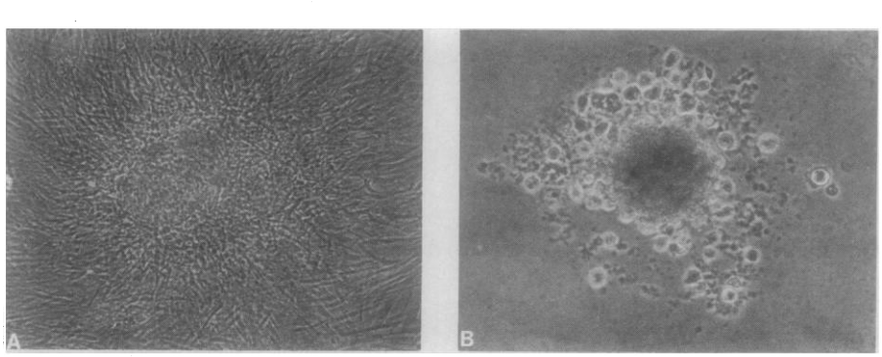


Fig. 1. Colony morphology of transformed Bloom's syndrome cells. (A) Typical focus of transformed Bloom's syndrome cells transfected with Ha-MuSV DNA. (B) Anchorage-independent colony derived from GM3498B-HV2–transformed cell line.

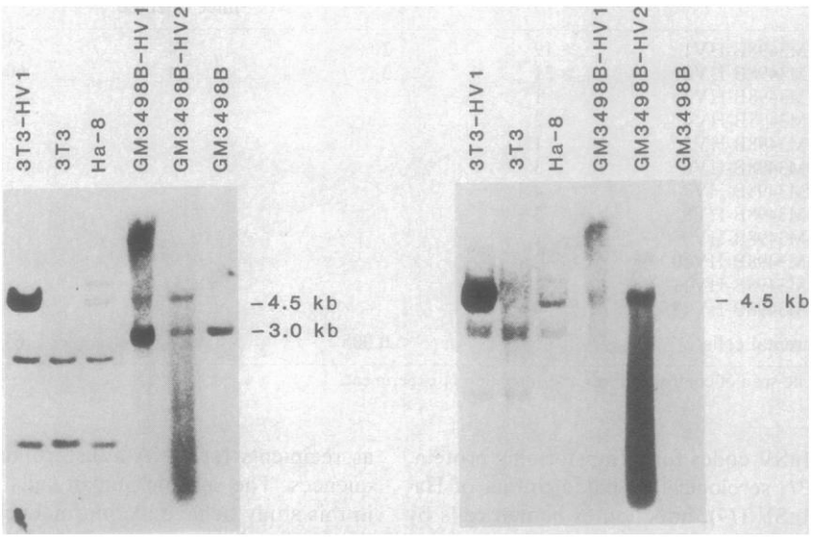
The transformed cell lines and the parental cells have a normal human chromosome constitution (46;XY) and the high frequency of sister chromatid exchange (SCE) (55 to 65 per cell) characteristic of Bloom's syndrome cells [Table 1 and (*25*)]. As often observed, two subpopulations with distinct SCE frequencies were present in each culture; one with 45 to 50 and another with 70 to 95 SCE's per cell. Therefore, the two transformed lines, GM3498B-HV1 and GM3498B-HV2, have retained the characteristic phenotype of a high spontaneous SCE level associated with Bloom's syndrome cells.

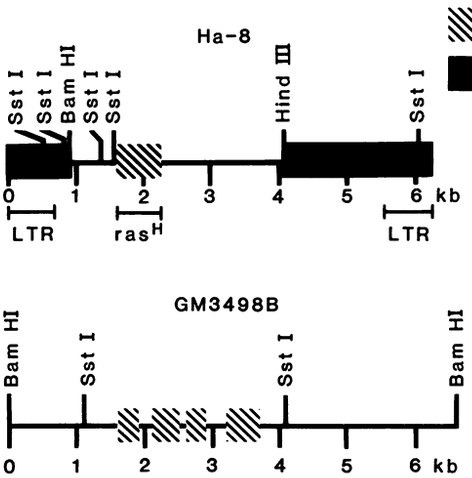
The transformed cell lines were examined by Southern blot analysis (*26*) to determine whether they have acquired the Ha-MuSV sequence. The blots were probed with v-*bas*, the mouse homolog of the Ha-MuSV oncogene (*11*), which also recognized the human ras^H homolog. Endonuclease Sst I was used to restrict the DNA to distinguish the normal v-*bas* human homolog from that of Ha-MuSV; Sst I digestion of human and Ha-MuSV DNA gives rise to a 3- and a 4.5-kb fragment, respectively, containing ras^H sequences (Fig. 2). The DNA's of the transformed lines GM3498B-HV1 and GM3498B-HV2 both have sequences of 4.5 and 3.0 kb homologous to v-*bas*, whereas parental cells have only the normal human 3.0-kb fragment. Furthermore, the 4.5-kb v-*bas* homologous sequence is observed in Ha-8 but not 3T3 DNA. As a positive control, DNA was included from 3T3-HV1, a 3T3 cell line derived by transfection with Ha-8 DNA. The large amount of the 4.5-kb ras^H sequence in 3T3-HV1 relative to Ha-8 probably reflects gene amplification that occurred during the transfection process. The data suggest that the Bloom's syndrome cells were transformed by acquiring the Ha-MuSV genome.

To confirm that the 4.5-kb fragment of the Bloom's syndrome transformants is the Ha-MuSV genome and not a fortuitous insertion into a 4.5-kb Sst I fragment, the DNA's were analyzed for Ha-MuSV sequences other than ras^H. The v-*bas* probe was eluted from the Southern blots, which were then probed with *p-14*, a fragment of the Ha-MuSV genome that contains the long terminal repeat (LTR). Both Bloom's syndrome cell transformants contain a 4.5-kb fragment hybridizing to *p-14*, whereas parental GM3498B DNA contains no sequences homologous to *p-14* (Fig. 2B). The DNA from all the mouse cells contained multiple bands homologous to *p-14* because multiple LTR's in mouse cell DNA exist in the genome as part of endogenous C-type viral DNA. Ha-8 and 3T3-HV1, however, contain an additional 4.5-kb fragment homologous to *p-14* at exactly the same position as the fragment that hybridizes to v-*bas*; again, because of many gene copies, 3T3-HV1 exhibits more hybridization with this 4.5-kb fragment. Therefore, most if not all, of the Ha-MuSV genome has been inserted into two transformed human cell foci. Furthermore, these data show conclusively that these transformed human cells had incorporated foreign DNA during transfection.

The presence of the Ha-MuSV genome in any one observed transformed line could be purely fortuitous because approximately 0.1 percent of the genome becomes incorporated into the recipient cells when genomic DNA is used as a donor in transfection experiments (*27*). The probability that any nonselected gene is present in both transformants is 10^{-6}. Therefore, it is highly probable that the Ha-MuSV genome is the transforming sequence.

Normal human cells have been neoplastically transformed by infection with whole oncogenic avian, murine, and primate RNA retroviruses, including Kirsten MuSV (*28–30*). Because Kirsten





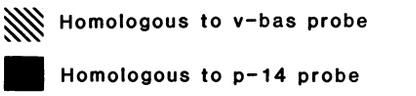


Fig. 2. Transformed Bloom's syndrome cells contain the Harvey virus genome. Sst I–digested DNA (25 μg) was subjected to electrophoresis in 0.8 percent agarose gels (*26*) and electroblotted (*36*) onto Gene Screen (New England Nuclear, Boston, Massachusetts). Hybridization with ^{32}p-labeled alkali denatured DNA probe was carried out for 16 hours at 42°C in 50 percent (by volume) formamide, 10 percent (weight to volume) dextran sulfate, 5× SSPE (saline, sodium phosphate, and EDTA), 10× Denhrardt's solution, and 1 percent (weight to volume) sodium dodecyl sulfate. After hybridization the filters were washed for 20 minutes at 60°C in 0.1× SSPE, and 0.1 percent sodium dodecyl sulfate. The restriction maps of DNA sequences in Ha-8 and in GM3498B human cells with regions homologous to v-*bas* are shown. Data for restriction sites in Ha-8 DNA are from Ellis *et al.* (*37*) and those for the *p-14* homologous sequence, LTR, and ras^H positions are from Ellis *et al.* (*38*); data for restriction sites in the human Bam HI 6.6-kb fragment with the v-*bas* homolog is from Tabin *et al.* (*15*). DNA sizes were determined from the positions of electrophoresed λ phage Hind III fragments. (A) Hybridization with the v-*bas* probe, a 0.6-kb Hind III–Bam HI (clone HB-l) fragment from plasmid pHB-1. HB-1 lacks homology to lytic retroviral sequences (*39*). (B) Probe was eluted for 30 minutes at 60°C in 96 percent (by volume) formamide, 10 m*M* EDTA, and 20 m*M* tris-HCl, *p*H 7.5. The filters were then hybridized with the *p-14* probe, a subcloned Hind III–Bam HI fragment (2.4 kb) from cloned Ha-MuSV DNA (*38*).

Table 1. Properties of transformed foci and parental Bloom's syndrome cells. Bloom's syndrome GM3498B cells (Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, New Jersey) were transfected with 75 μg of DNA from Ha-8 (Ha-MuSV–transformed 3T3 cells). Cultures (14th passage) were trypsinized and 3×10^5 cells were placed in 35-mm dishes. After 24 hours, the cells were fed and incubated for an additional 24 hours. The calcium phosphate coprecipitation procedure was similar to that described (*32, 33*). Cultures (five dishes) were incubated with 15 μg of DNA per dish for 7 hours. After 5 weeks of incubation, 12 foci were observed, isolated, and characterized. To determine frequency of colony formation, cells (10^4 per 60-mm dish) were placed in agarose medium (*22*) and incubated for 3 weeks; the foci were counted and their frequency was determined. The frequency of neurological abnormalities was determined by injecting nude mice intracranially with 5×10^5 cells into the right parietal subcortical region (*34*). Mice that did not develop neurological abnormalities after injection of cells had a normal life-span and showed no abnormal brain histology. Sister chromatid exchange frequencies of exponentially growing cultures were determined after fluorescence-Giemsa staining (*35*). The chromosomes of 25 cells were examined for each point; data are means ± standard errors.

Focus	Life-span (passages)	Frequency of colony formation in agarose ($\times 10^4$)	Ratio of mice with neurological motor dysfunction and death to mice injected	SCE per cell
GM3498B-HV1	> 19	2	2/4	57 ± 3
GM3498B-HV2	> 21	3	3/4	60 ± 3
GM3498B-HV3	1			
GM3498B-HV4	2			
GM3498B-HV5	1			
GM3498B-HV6	3			
GM3498B-HV7	4			
GM3498B-HV8	3			
GM3498B-HV9	2			
GM3498B-HV10	2			
GM3498B-HV11	1			
GM3498B-HV12	4			
Parental cells	5*	< 0.005	0/4	63 ± 4

*Life-span of control cultures after initiation of experiment.

MuSV codes for a transforming protein, *p21*, serologically related to that of Ha-MuSV (*19*), infection of human cells by Ha-MuSV would be expected to result in transformation. Our results suggest that the Ha-MuSV genome does transform human cells by transfection. Thus, the genomes of oncogenic retroviruses including Ha-MuSV can serve as complete DNA-transforming sequences for human cells.

The information acquired from the use of rodent cells for transfection with DNA DNA-transforming sequences, although important for elucidating gene action in rodent cells, may not be entirely applicable to human biology. Importantly, human cells are not easily transformed to the malignant state (*31*). Furthermore, there are no reports of transfection experiments in which human cells are used as recipients for DNA-transforming sequences. The specific human cells used in this study behave like normal cells in that they maintain a stable diploid karyotype and finite life-span in culture. However, these cells from a patient with Bloom's syndrome have abnormal genetic attributes, including a high spontaneous sister chromatid exchange frequency, which may be facilitating DNA transfection. The use of human cells such as these may be useful for a complete analysis of the molecular control of human cancer.

J. DONIGER
J. A. DI PAOLO
N. C. POPESCU

Laboratory of Biology, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland 20205

References and Notes

1. C. Shih, B. Z. Goldfarb, M. P. Danneberg, R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5714 (1979).
2. G. M. Cooper and P. E. Neiman, *Nature (London)* **287**, 656 (1980).
3. C. Shih, L. C. Padhy, M. Murray, R. A. Weinberg, *ibid.* **290**, 261 (1981).
4. M. A. Lane, A. Sainten, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5185 (1981).
5. _____, *Cell* **28**, 873 (1982).
6. M. Perucho, M. Goldfarb, K. Shimizu, C. Lama, J. Fogh, M. H. Wigler, *ibid.* **27**, 467 (1981).
7. N. Hopkins, P. Besmar, A. B. DeLeo, L. A. Low, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7555 (1981).
8. S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, K. C. Robbins, M. Barbacid, *ibid.* **79**, 2845 (1982).
9. S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, S. A. Aaronson, M. Barbacid, *Nature (London)* **300**, 539 (1982).
10. K. Shimizu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2112 (1983).
11. E. Santos, S. R. Tronick, S. A. Aaronson, S. Pulciani, M. Barbacid, *Nature (London)* **298**, 343 (1982).
12. L. F. Parada, C. J. Tabin, C. Shih, R. A. Weinberg, *ibid.* **297**, 474 (1982).
13. M. Goldfarb, K. Shimizu, M. Perucho, M. Wigler, *ibid.* **296**, 404 (1982).
14. C. Shih and R. A. Weinberg, *Cell* **29**, 161 (1982).
15. C. J. Tabin *et al.*, *Nature (London)* **300**, 143 (1982).
16. E. P. Reddy, R. K. Reynolds, E. Santos, M. Barbacid, *ibid.*, p. 149.
17. E. Taparowski, Y. Suard, O. Fasano, K. Shimizu, M. Goldfarb, M. Wigler, *ibid.*, p. 762.
18. J. German, *Chromosome and Cancer*, J. German, Ed. (Wiley, New York, 1974), p. 601.
19. T. Y. Shih, M. O. Weeks, H. A. Young, E. M. Scolnick, *Virology* **96**, 64 (1979).
20. E. M. Scolnick, personal communication.
21. J. Doniger, J. A. Di Paolo, N. C. Popescu, unpublished data.
22. A. I. Neugut and I. B. Weinstein, *In Vitro* **15**, 351 (1979).
23. I. MacPherson and L. Montagnier, *Virology* **23**, 291 (1964).
24. C. H. Evans and J. A. Di Paolo, *Cancer Res.* **35**, 1035 (1975).
25. R. S. Chaganti, S. Schonberg, J. German, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4508 (1974).
26. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
27. M. Perucho, D. Hanahan, M. Wigler, *Cell* **22**, 309 (1980).
28. F. C. Jensen, A. J. Girardi, R. V. Gilden, H. Koprowski, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 53 (1964).
29. S. A. Aaronson and G. J. Todaro, *Nature (London)* **225**, 458 (1970).
30. S. A. Aaronson, *Virology* **52**, 562 (1973).
31. J. A. Di Paolo, *J. Natl. Cancer Inst.* **70**, 3 (1983).
32. F. L. Graham and A. J. van der Eb, *J. Virol.* **52**, 456 (1973).
33. M. Wigler, S. Silverstein, L. S. Lee, A. Pellicer, Y. Cheng, R. Axel, *Cell* **11**, 223 (1977).
34. J. W. Greiner, C. H. Evans, J. A. Di Paolo, *Carcinogenesis* **4**, 359 (1981).
35. B. S. Emanuel, *Am. J. Hum. Genet.* **30**, 153 (1978).
36. M. Bittner, P. Kupferer, C. F. Morris, *Anal. Biochem.* **102**, 459 (1980).
37. R. W. Ellis *et al.*, *J. Virol.* **36**, 408 (1980).
38. R. W. Ellis *et al.*, *Nature (London)* **292**, 506 (1981).
39. P. R. Andersen, S. R. Tronick, S. A. Aaronson, *J. Virol.* **40**, 431 (1981).
40. We thank E. M. Scolnick for Ha-8 cells, S. R. Tronick for pHB-1, and D. R. Lowy for *p-14*. We also thank A. J. Demarinis and S. Amsbaugh for technical assistance and S. R. Tronick and D. R. Lowy for discussions.

5 July 1983; accepted 3 October 1983