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Enhancing Effect of Murine Leukemia Virus on Fibroblast Transformation by Normal BALB/C Mouse DNA Fragments

K. J. van den Berg, V. Krump-Konvalinkova, and P. Bentvelzen

A. Introduction

Several rapidly transforming RNA tumor viruses contain inserts of cellular genetic material. These inserts seem to constitute the so-called *onc* genes of these viruses (Stephenson et al. 1979). The question arises whether during the recombination process leading to the generation of highly oncogenic viruses the cellular sequences become altered in such a way that they will cause malignant transformation. This would then be compatible with simplistic somatic mutation theories of nonviral carcinogenesis. Alternatively, with the virus acting as a vector, the insertion of normal cellular sequences involved in control of growth and/or differentiation into other sites of the host genome may lead to overproduction of certain gene products and eventually to malignant transformation. This transposition phenomenon may have important implications for models of nonviral carcinogenesis.

B. Transformation by Normal Cell DNA

Recently Cooper et al. (1980) reported that mechanically fragmented normal cellular DNA isolated from avian or mammalian cell lines could transform NIH/3T3 cells, albeit at a low frequency. Secondary transfections with DNA isolated from the transformed cells proved to be highly efficient.

During our study on transfection with the integrated provirus of Abelson murine leukemia virus (A-MuLV) utilizing the calciumtechnique of Graham and van der Eb (1973), we observed independently of Cooper et al. in control experiments that mechanically fragmented DNA isolated from the thymus of normal BALB/c mice could transform NIH/3T3 as well as BALB/3T3 cells. In contrast to the foci of transformed cells induced by the A-MuLV provirus which appear within 2 weeks, those induced by normal DNA were first observed after 4 weeks, corresponding with six subcultures of the recipient cell.

The DNA specificity can be concluded from Table 1 in which treatment of the DNA preparation with DNase abrogated fully the transforming capacity, while RNase or pronase did not. Neither calf thymus nor *E.coli* DNA induced foci of transformed cells.

Since DNA isolated from thymus, spleen, or liver had equal transforming capacity (see Table 2), the transforming sequences seem to be an integral part of the BALB/c genome. As DNA isolated from germfree BALB/c mice of a colony established 20 years ago is similarly transforming, this property cannot be due to recent infection of the germ line with an oncogenic virus.

C. Characterization of Transformed Cell Lines

A battery of cell lines were developed from foci of transformants induced by normal BALB/c DNA fragments. No reverse transcriptase activity could be detected in any of the cell lines, indicating that transformation occurred in the absence of replicating retroviruses.

The cells grew well in soft agar, indicating anchorage independence. Upon intraperitoneal inoculation into newborn athymic BALB/ nude mice, all of six tested cell lines were oncogenic. After subcutaneous injection of 10^6 cells into 3-week-old BALB/c five of six lines produced fibrosarcomas within 3 weeks.

Table 1. Transformation of 3T3 cells by normal BALB/c DNA

	Recipient cells			
	BALB/3T3		NIH/3T3	
	Fraction transformed cultures	Average No. foci/dish	Fraction transformed cultures	Average No. foci/dish
BALB/c thymus DNA 50 $\mu g \cdot ml^{-1}$	16/16	15	16/16	4
Ibid, treated with pronase[a]	8/8	4	7/8	1.5
BALB/c thymus DNA 50 $\mu g \cdot ml^{-1}$ RNase[a]	8/8	2	8/8	6
BALB/c thymus DNA 50 $\mu g \cdot ml^{-1}$ DNase[a]	0/8	0	0/8	0
BALB/c thymus DNA 50 $\mu g \cdot ml^{-1}$ heat denaturated[b]	3/8	0.5	3/8	0.5
Calf thymus DNA, 50 $\mu g \cdot ml^{-1}$	0/8	0	0/8	0
E. coli DNA, 50 $mg \cdot \mu l^{-1}$	0/8	0	0/8	0

[a] 1 h at 37°C
[b] 5 min at 100°C, then quickly placed in ice

So far, no transforming virus could be rescued after infection with the Moloney strain of MuLV (Mo-MuLV) in contrast to cells transformed by A-MuLV proviral DNA. A hyperimmune antiserum, raised in C57BL against syngeneic A-MuLV induced lymphoma cells and absorbed with syngeneic Mo-MuLV lymphoma cells, reacted with two of seven testet cell lines transformed by normal DNA in the cytoplasmic immunofluorescence test. The antiserum reacted with a variety of A-MuLV transformed cells but not with NIH/3T3 cells infected with Mo-MuLV.

D. Enhancement of Transformation by Preinfection with Leukemia Virus

The efficiency of transformation of normal cell DNA is rather low. Since the nontransforming murine leukemia viruses make cells susceptible to the action of various carcinogenic chemicals (Huebner and Gilden 1972), we studied the influence of preinfection with MuLV-strains in this system. After incubation of NIH/3T3 cells, infected with either Rauscher or Moloney-MuLV, with normal cell DNA fragments, foci could be detected within 2 weeks after a single passage. The transformation frequency could be as high as 1 focus forming unit per 1 ug DNA (see Table 3), which would be comparable to the results obtained with the A-MuLV provirus. A linear dose-response relationship was found in this system (Fig. 1), indicating that incorporation of a single DNA fragment would be sufficient for neoplastic conversion. Quite remarkable is that a minimal quantity of DNA is needed for transformation to occur.

E. Discussion

It is unlikely that the transforming capacity of normal cell DNA is due to sheer mutagenicity, since mouse DNA sequences homologous to the *src* gene of Moloney murine sarcoma virus cannot transform fibroblasts (Oskarsson et al. 1980). Our data and those of Cooper et al. (1980) support the notion that the cellular

Table 2. Effect of origin of BALB/c DNA on transforming activity[a]

Source of DNA		Recipient cells (Fraction transformed cultures)	
		BALB/3T3	NIH/3T3
Thymus	50 $ug \cdot ml^{-1}$	16/16	16/16
	25 $ug \cdot ml^{-1}$	8/8	N.D.
Spleen	50 $ug \cdot ml^{-1}$	8/8	N.D.
	25 $ug \cdot ml^{-1}$	8/8	2/8
Liver	50 $ug \cdot ml^{-1}$	4/4	N.D.

[a] N.D. = not done

Table 3. Effect of preinfection with murine leukemia virus on early transformation by normal BALB/c DNA

Recipient cells	Treatment	Fraction transformed cultures at 2 weeks	Average no. foci/dish	Average transformation freq. ($\times 10^6$/ug DNA/cel)
NIH/3T3	BALB/c liver DNA[a]	0/33	0	0
	E. coli DNA	0/33	0	0
	transfection buffer	0/33	0	0
NIH/3T3-R-MuLV[b]	BALB/c liver DNA	3/3	4	2
	E. coli DNA	0/3	0	0
	transfection buffer	0/3	0	0
NIH/3T3-Mo-MuLV[c]	BALB/c liver DNA	33/33	18	7
	E. coli DNA	0/33	0	0
	transfection buffer	0/33	0	0

[a] 25 ug · ml^{-1}
[b] NIH/3T3 infected with Rauscher murine leukemia virus 4 days before transfection
[c] NIH/3T3-cloned after having been productively infected with Moloney murine leukemia virus

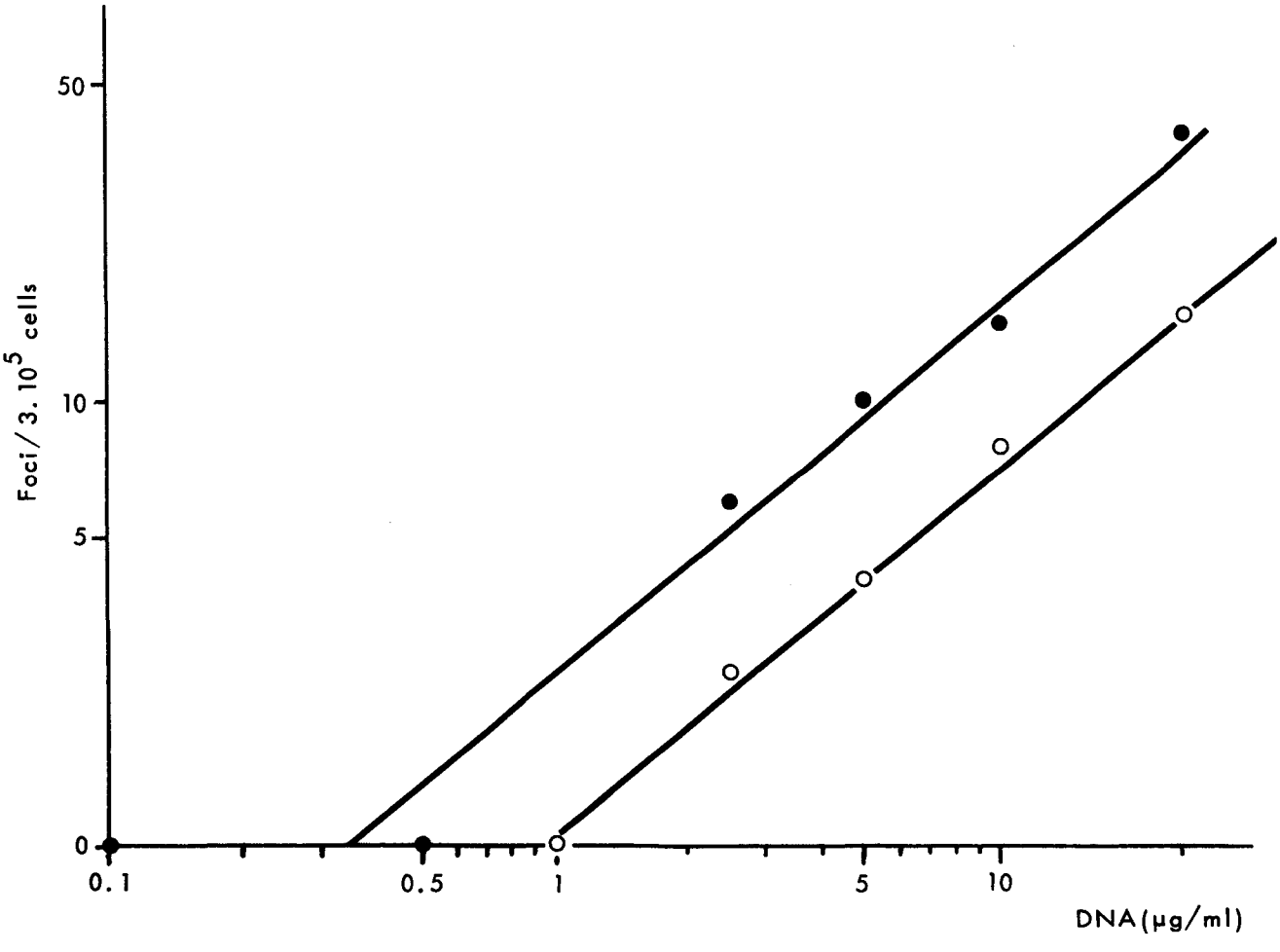

Fig. 1. Kinetics of transformation of NIH/3T3 cells infected with Mo-MuLV by fragmented normal BALB/c DNA

inserts in the genome of rapidly transforming viruses have not been perturbated. They suggest that transposition of certain cellular genes may result in oncogenic transformation. It has been hypothesized that this was due to abrogation of negative control by neighboring genes (Van Bekkum 1975). Alternatively, and more likely, it may be due to the insertion of the cellular genes close to the active promotor sequences. The latter hypothesis would explain the low efficiency of primary transfections and the better transforming rate of secondary ones.

The immunofluorescence staining of some of the transformed lines with an antiserum presumably directed against the *onc* gene of A-MuLV suggest that in these cases the homologous gene might have been incorporated at a different site resulting in transformation. Several other cellular *onc* genes must have been involved in the generation of the other transformed cell lines.

The enhancing effect of murine leukemia virus may be due to the induction of a semitransformed state of the 3T3 cells by MuLV, which would explain the single-hit transformation by normal cellular DNA fragments. Another hypothesis, which we favor at the moment, is that MuLV provides highly active promotor sequences. This is supported by the finding that the complete provirus of Moloney murine sarcoma virus, which contains leukemia virus derived promotors, is not enhanced in its transforming capacity by preinfection of recipient cells with MuLV (Anderson et al. 1979).

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References

Anderson P, Goldfarb MP, Weiberg RA (1979) A defined subgenomic fragment of in vitro synthesized moloney sarcoma virus DNA can induce cell transformation upon transfection. Cell 16:63–75 – Cooper GM, Okenquist S, Silverman L (1980) Transforming activity of DNA of chemically transformed and normal cells. Nature 284:418–421 – Graham FL, van der Eb AJ (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52:456–467 – Huebner RJ, Gilden RV (1972) Inherited RNA viral genomes (virogenes and oncogenes) in the etiology of cancer. In: Emmelot P, Bentvelzen P (eds) RNA viruses and host genome in oncogenesis. North-Holland, Amsterdam, pp 197–21g – Oskarsson M, McClements WL, Blair PG, Maizel JV, van de Woude GF (1980) Properties of a normal mouse cell DNA sequence (sarc) homologous to the src sequence of Moloney sarcoma virus. Science 207:1222–1224 – Stephenson JR, Khan AS, van de Ven WJN, Reynolds FH Jr (1979) Type C retroviruses as vectors for cloning cellular genes with probable transforming function. J Natl Cancer Inst 63:1111–1119 – Van Bekkum DW (1975) Mechanisms of radiation carcinogenesis. In: Nygaard OF, Adler HI, Sinclair WK (eds) Radiation research biomedical, chemical and physical perspectives. Academic Press, New York, pp 886–894