HORMONAL REGULATION OF v-src ONCOGENE EXPRESSION IN NIH 3T3 MOUSE FIBROBLASTS

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Tumor formation in vivo is a multistage process. The multistage nature of carcinogenesis also has been demonstrated on models of neoplastic conversion of cells in culture. The transformed cell differs from the normal in several parameters: morphology, dependence of growth on serum factors, independence of growth on the substrate, tumorigenicity for animals, and so on. Analysis of a large number of tumors and lines of transformed cells has shown that at some stages oncogenes are involved in the process of carcinogenesis [2, 9, 10]. Experiments with infection of cells by temperature-sensitive (TS) mutants of transforming viruses have shown that to maintain the transformed phenotype, the oncogene must be constantly expressed in the cells. If synthesis of the oncogene product falls below a certain level, the cells will revert to the normal phenotype [7]. At least two possibilities exist for the experimental regulation of oncogene action in cells: 1) the use of TS mutants of oncogenic viruses; 2) the use of recombinant plasmids containing oncogenes whose transcription is controlled by regulatory promoters. It has been shown, for instance, that mutants of viruses partially defective for transformation can induce the appearance only of some of the characteristic parameters of transformed cells in the infected cells [13]. However, the most interesting approach is evidently the use of recombinant plasmids containing oncogenes under promoter control, for these models can be used to investigate the effect of oncogene product dose on manifestation of the various parameters of transformation. This approach thus enables an experimental model of controlled multistage cell transformation to be obtained in

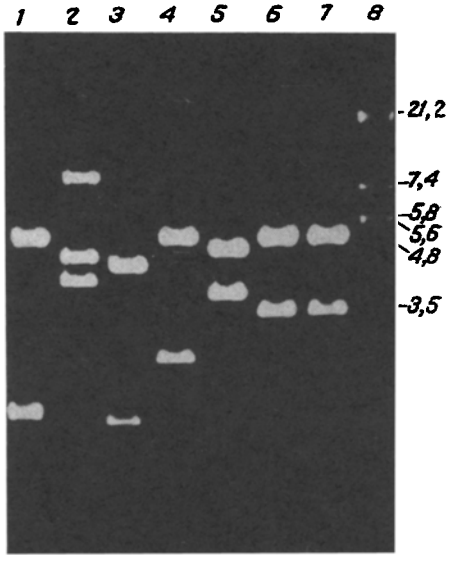

Fig. 1. Electrophoretic fractionation of restriction fragments of DNA of plasmid pMLsrc 10 in 0.8% agarose. 1) EcoRI + BglII 2) SalGI, 3) PstI, 4) EcoRI + XhoI, 5) BamHI, 6) HindIII, 7) EcoRI, 8) λcI + EcoRI.

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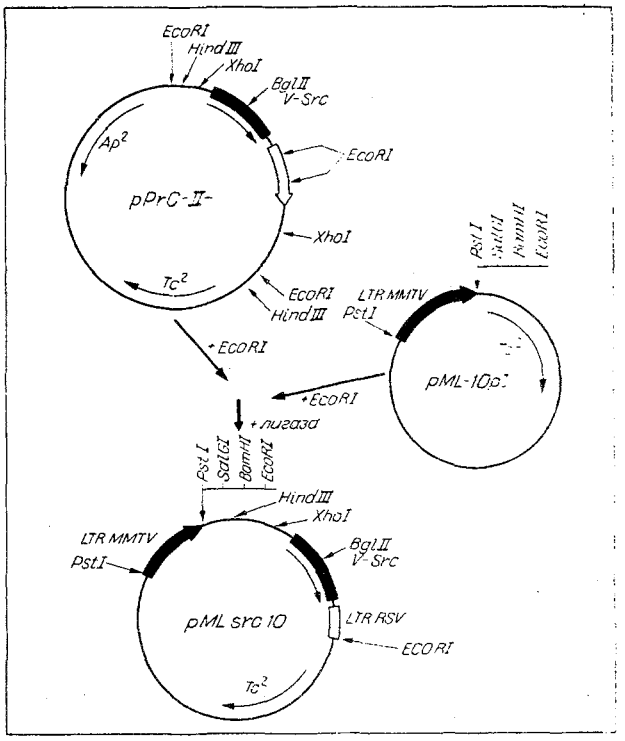

Fig. 2. Diagram of method of obtaining plasmid pMLsrc 10.

TABLE 1. Formation of Foci of Morphologically Transformed NIH 3T3 Cells after Transfection with Plasmid pMLsrc 10 and pEJras 6.6

Plasmid	Presence of dexamethasone in medium	Efficiency of transformation, FFU/μg plasmid DNA
pMLsrc10	—	12—14
pMLsrc10	+	120—130
pEJras 6.6	—	16—22
pEJras 6.6	+	15—20

vitro. Recombinant plasmids containing an oncogene under hormone-dependent LTR promoter of mouse mammary tumor virus (MMTV) and the promoter of mouse metalloprotein 1 (MT-1) gene are nowadays used in investigations of this kind. Transcription activity of the LTR MMTV promoter is sharply increased on interaction of the glucocorticoid hormone—receptor complex with specific sequences located before the promoter [3]. Transcription activity of mouse MT-1 gene promoter depends on the presence of Zn^{++} and Cd^{++} cations. Plasmids containing v-ras [6], v-mos [4, 11], and v-src [8] oncogenes and the middle T-antigen of polyoma virus [12], whose expression was regulated by LTR MMTV, and also a plasmid containing v-src under the promoter-controlled mouse MT-1 gene [5], were obtained. Mouse and rat fibroblasts of various lines were transfected with these plasmids. Manifestation of the transformed properties of the cell was enhanced in all the systems tested with an increase in the dose of oncogene product in them.

The aim of this investigation was to study the effect of dexamethasone on transforming activity of a constructed plasmid containing the v-src oncogene under LTR MMTV promoter control.

EXPERIMENTAL METHOD

Materials. Restriction endonucleases were obtained from the "Ferment" Research and Production Combine (Vilnius). α-^{32}P-TTP (1000 Ci/mmole) was prepared by the "Izotop" All-Union Combine (Tashkent). Agarose for electrophoresis was type II, from "Sigma" (USA). Nitrocellulose filters were obtained from "Schleicher und Schüll" (West Germany).

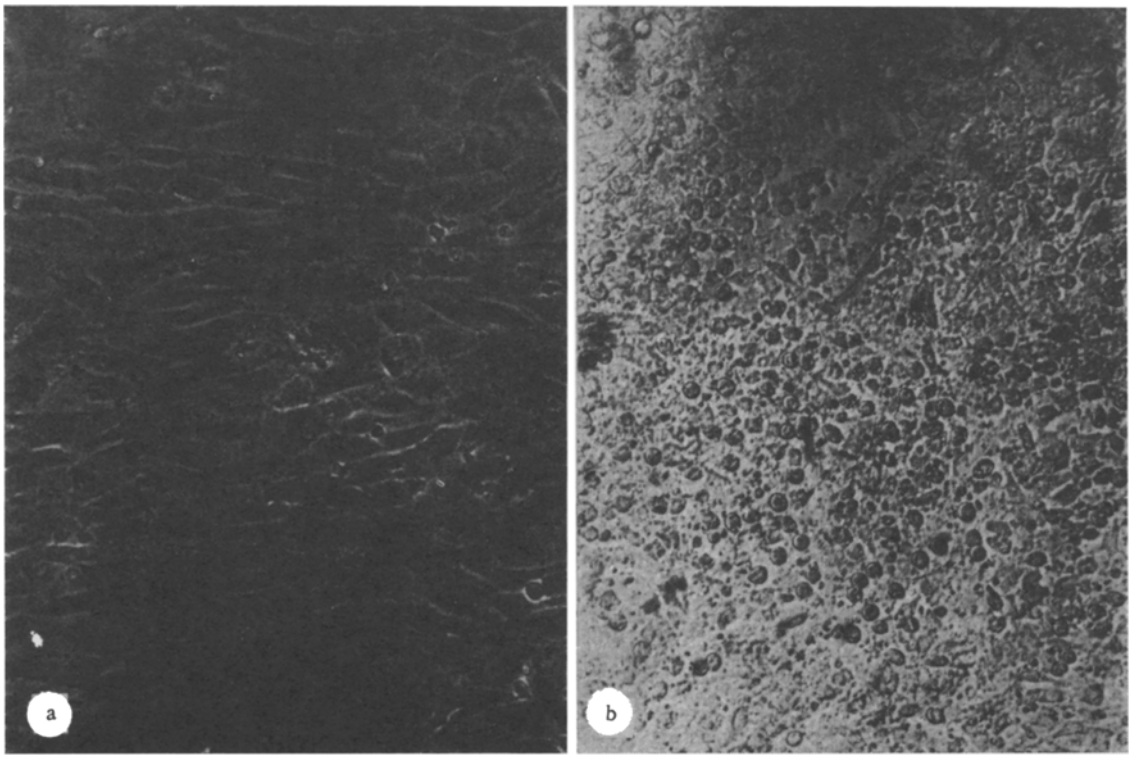

Fig. 3. Morphological transformation of NIH 3T3 cells after transfection with plasmid pMLsrc 10 (phase contrast, 50 ×). a) Normal NIH 3T3 cells; b) NIH 3T3 cells after transfection with plasmid pMLsrc 10.

Plasmids. The PstI-fragment from DNA of phage λ MMTV-GP, containing the LTR MMTV sequence, was generously provided by Dr. E. Buetti (Switzerland), and was recloned into pBR 322 (pML-10). The Pst—EcoRI fragment containing pBR 322 sequences was deleted from the plasmid thus obtained and a polylinker from plasmid pUC-K (pMV-10 pl) was introduced. Plasmid pPrC-il was generously provided by Dr. A. G. Tatosyan (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). pPrC-11 contained some sequences from the circular provirus RSV-PrC, cloned relative to the HindIII site in pBR 322 [1]. Plasmid pEJras 6.6 was generously provided by Dr. R. Weinberg, USA.

DNA analysis. High-molecular-weight DNA was extracted from tumors arising in nude mice after injection of NIH 3T3 cells, transformed by plasmid pMLsrc 10, digested by restriction enodnucleases, and analyzed by the Southern blotting method. Nick-translated HindIII-EcoRI fragments, containing the structural part of v-src and part of the U_3LTR RSV region, were used for hybridization of the filters.

Transfection. NIH 3T3 cells were used for transfection. The cells were subcultured 20 h before transfection at the rate of $2 \cdot 10^5$-$3 \cdot 10^5$ to 60 mm in a Petri dish in medium MEM (from "Serva" or made in Czechoslovakia) with 10% embryonic calf serum. Transfection was carried out by the calcium phosphate precipitate method [15]. Plasmid DNA (2.5 μg), mixed with 10 μg of salmon sperm high-molecular-weight DNA as coprecipitator, after precipitation with calcium chloride, was applied to a 70% cell monolayer and incubated for 4 h at 37°C in medium with CO_2. After incubation the medium was changed for one with 5% embryonic serum and with (or without) dexamethasone (10^{-6} M). The cells were maintained on dishes for 14 days, with the medium changed periodically.

Tumorigenicity of the transformed cells was studied in nude mice, generously provided by Dr. E. S. Revazova (All-Union Oncologic Research Institute, Academy of Medical Sciences of the USSR).

EXPERIMENTAL RESULTS

Construction of the Recombinant Plasmid pMLsrc 10. The PstI fragment from the left side of the integrated MMTV covirus contained LTR sequences besides 5-10 base pairs located at the 5'-end of the provirus. The cap site of MMTV RNA was matched in the 271 nucleotide

position above the 3'-end of the PstI-fragment. An EcoRI-fragment from plasmid pPrC-11, containing structural part of the v-src oncogene, the U_3 region of LTR rsv and the HindIII-EcoRI fragment of pBR 322 were inserted into the resulting pML-10 pl vector at the EcoRI site (Figs. 1 and 2). Thus recombinant plasmid pMLsrc 10, used in experiments on transfection of NIH 3T3 cells, contained the structural part of the v-src oncogene in the forward orientation relative to LTR MMTV.

The plasmid has a polyadenylation site and transcription terminates in the U_3 region of the right LTR-provirus of rsc [14].

Transfection of NIH 3T3 Cells by Plasmid pMLsrc 10. NIH 3T3 cells were transfected with DNA of plasmid pMLsrc 10. After transfection the cells were maintained on growth medium with 5% embryonic calf serum with or without dexamethasone. DNA of plasmid pEJras 6.6 and salmon sperm DNA were used as the controls for transfection of these same cells. Morphological transformation of NIH 3T3 cells was found two weeks after transfection with plasmid pMLsrc 10 — against the background of a fibroblast monolayer foci of round, refractile cells appeared (Fig. 3). Morphologically changed cells were found on dishes with and without dexamethasone, at the same time, although the size of the transformation foci and their number depended on the presence of dexamethasone in the medium. Foci of transformed cells obtained after transfection with DNA of plasmid pMLsrc 10 on dishes with dexamethasone were smaller, and the transformed cells were more easily detached from the substrate, and the number of foci was an order of magnitude greater than on dishes without dexamethasone (Table 1). The number of foci of transformation of NIH 3T3 cells, transfected with pMLsrc 10 DNA in the absence of dexamethasone was similar to the number of foci obtained on transfection of these same cells by DNA of plasmid pEJras 6.6, and the presence of dexamethasone led to a tenfold increase in the efficiency of morphological transformation, but did not affect the efficiency of transformation of the cells by plasmid pEJras 6.6 (Table 1).

After the appearance of morphological changes as a result of transfection with plasmid pMLsrc 10 the NIH 3T3 cells were subcultured and injected in a dose of $0.4 \cdot 10^6$-$1 \cdot 10^6$ per animal into nude mice. After 2-3 weeks tumors were found at the site of the injection in the mice, and the times of their appearance did not depend on whether the cells had been cultured after transfection in medium with or without dexamethasone, but were determined by the number of cells injected.

Thus the plasmid obtained, containing the v-src oncogene under LTR MMTV promoter control, can induce transformation of NIH 3T3 cells in both the presence and absence of dexamethasone. The presence of dexamethasone in the medium increased the efficiency of cell transformation tenfold, but tumorigenicity was unchanged under these circumstances. Similar results were obtained by Jakobovits et al. [8]. They showed that the levels of endogenous steroid hormones induce an almost maximal level of pp60 v-src expression under LTR MMTV control.

By the use of the blot hybridization method v-src sequences were found (not shown) in high-molecular-weight DNA obtained from tumors induced in nude mice by NIH 3T3 cells, transformed by pMLsrc 10.

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