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Interaction of Promoters and Oncogenes During Transfection

L. Z. Topol, A. G. Tatosyan, and F. L. Kisseljov

DNA transfection in recipient NIH3T3 mice cells is most widely used for identification of transforming genes in human tumor cells [1]. We showed earlier that c-Ha-*ras* proto-oncogene from a human malignant glioma cell line (U 251 Mg) was active in transfection experiments. In primary U 251 Mg cells, c-Ha-*ras* gene was present in a single copy but amplified in transfected NIH3T3 cells [2].

For the purpose of further analyzing this system, we studied the influence of exogenous promoters on molecular-genetic processes which occur during transfection of genetic material. As a source of promoter, we used pLTR 1.5 plasmid which was subcloned from pPR c11 plasmid containing Rous sarcoma virus (RSV) provirus [3]. The pLTR 1.5 clone contained the intact LTR of RSV and the 5′ leader sequence of the viral genome.

This plasmid was cotransfected in NIH3T3 cells with DNA containing amplified c-Ha-*ras* from nude mice tumors developed as a result of the injection of the cells transfected by DNA from a human malignant glioma cell line [2] or with DNA from the normal NIH3T3 cells. In both cases, 7 days after cultivation in vitro, the cells (2×10^6) were injected into nude mice. In the first case, tumors developed in a 2-week period, and in the second one a little later. DNA from these cells was analyzed for LTR sequences and the activation of cell proto-oncogenes by molecular hybridization tests [4] using nick-tranlated LTR and oncogene probes [5]. Where cotransfection included pLTR 1.5 and DNA from NIH3T3 cells in the DNA of nude mice, the size of LTR-con-

Fig. 1. The analysis of pLTR 1.5 sequences in the genome of nude mice tumors induced by the injection of NIH3T3 cells transfected by various DNA preparations. *1* NLTR 1 (first round); *2* NLTR 2 (first round of the second experiment); *3* NLTR 1-1 (second round); *4* NLTR 1-2 (second round of the second experiment); *5* NIH3T3. Restriction with *Eco*RI. Washing conditions: 65 °C 0.1 × SSC, 0.1% SDS. Probe: ^{32}P-pLTR 1.5

Institute of Carcinogenesis, Cancer Research Center, Moscow, USSR

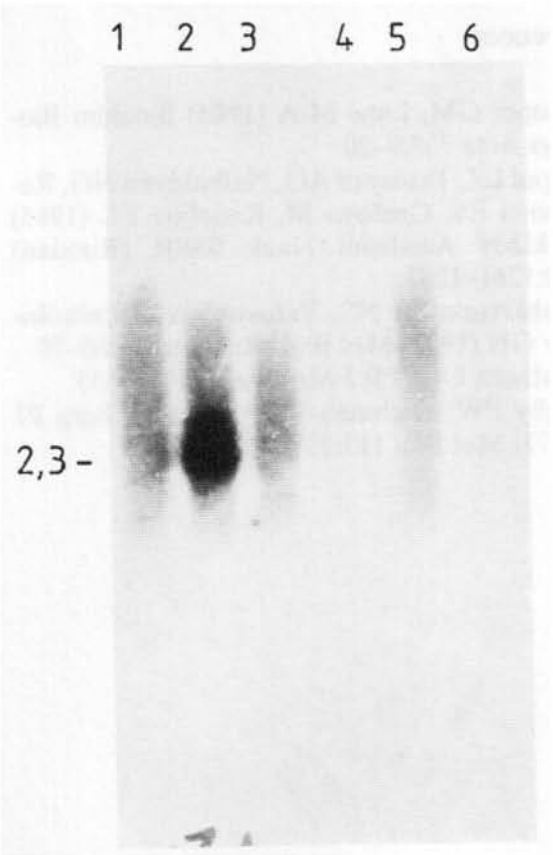

Fig. 2. Sequences specific for *fos* in RNA of nude mice tumors induced after the injection of NIH3T3 cells transfected by various DNAs. *1* NIH 3T3; *2* NLTR 1 (first round); *3* NLTR 1-1 (second round); *4* NMg 1-1 (second round); *5* NMg LTR 1 (first round); *6* NMg LTR 1-1 (second round). Probe: ^{32}P-*fos*

taining fragments was different in the first and the second rounds of transfection (Fig. 1).

On the basis of these data, we suppose that the integrated LTR can activate one of the cell proto-oncogenes. We tested several oncogenes (*fes, src, myc,* Ha-*ras, sis, abl, mos, fos*) and found that in this type of tumors c-*fos* oncogene transcription takes place. According to the results of Northern blotting, this RNA was represented by a 2.3-kb transcript (Fig. 2). The data obtained from blot hybridization with DNA of the tumors restricted with *Eco*RI and *Bam*HI indicate that no structural rearrangements in the c-*fos* gene were found.

Injection into nude mice of NIH3T3 cells cotransfected by pLTR 1.5 and DNA with amplified human c-Ha-*ras* gene also resulted in the development of tumors, which were designated as N MgLTR. The tumors also appeared in further rounds of transfection. In the genome of these tumors, the integration of LTR and pBr 322 plasmid sequences was shown (Fig. 3); the integrated site was localized in the plasmid region because after *Eco*RI restriction, an internal LTR RSV 0.3-kb fragment could also be detected. This is confirmed by Pst I digestion. Blot analysis showed that the integrated pLTR 1.5 se-

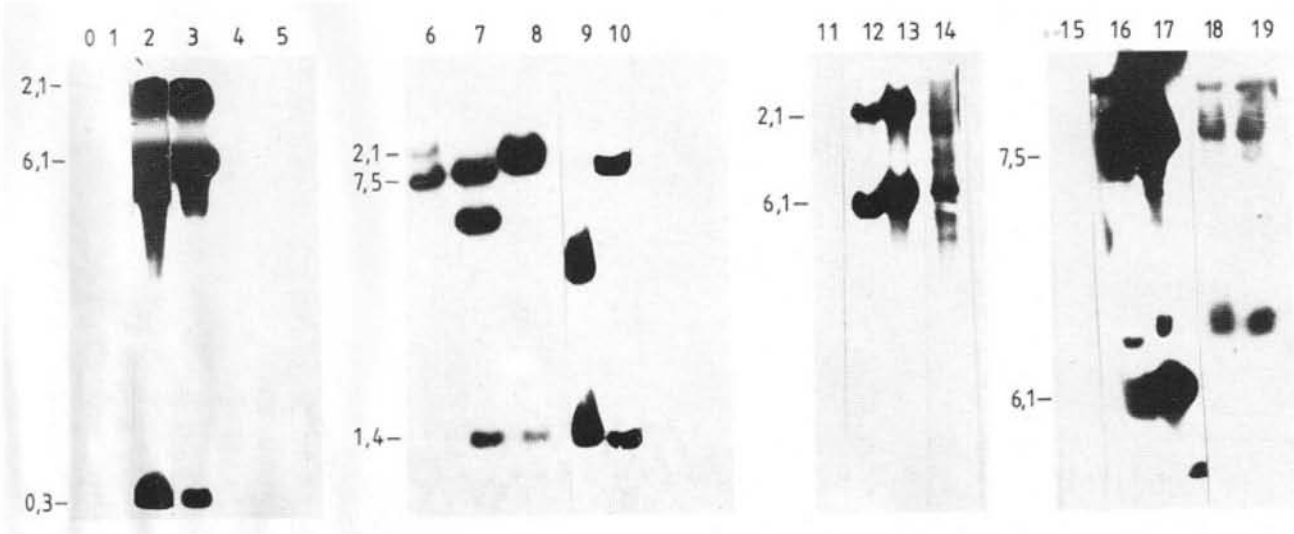

Fig. 3. Analysis of the genome of nude mice tumors – NMg LTR1 (first round) and NMg LTR 1-1 (second round). DNA preparations: *0* NLTR 1 (first round); *1, 11, 15* NIH3T3; *2, 6, 7, 8, 16* NMg LTR 1 (first round); *14* Np EJ-*ras* (nude mice tumor after transfection pEJ-*ras*-plasmid); *3, 10, 13, 17* NMg LTR (second round); *4* NMg 1 (first round); *5, 18* NMg 1-1 (second round); *9* pLTR 1.5; *16* NMg 1-1-1 (third round). Restriction; *0–5, 11–14 Eco*RI; *6 Bam*HI; *7 Bam*H 1 + Pst 1; *8–10, 15–19* Pst1. Probes: *0–10* ^{32}P pLTR 1.5; *11–14* ^{32}P-pBr 322; *15–19* ^{32}P EJ-*ras*

quences in the genome of tumor cells were amplified (Fig. 3) in comparison to NLTR tumor DNA. The status of amplification is preserved for the human Ha-*ras* oncogene as well. We suppose that the integrated pLTR 1.5 and Ha-*ras* sequences are associated in one and the same locus of cell genome. Thus, LTR cotransfection with two different types of mouse DNA (tumor and normal) is accompanied by various molecular-genetic events, but the final result is the acquisition of oncogenic potential by NIH3T3 cells.

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