EXPRESSION AND INTEGRATION OF EXOGENOUS DNA SEQUENCES TRANSFECTED INTO MAMMALIAN CELLS

F. COLBERE-GARAPIN1, M.L. RYHINER1 & A.C. GARAPIN2

1: Unité de Virologie Médicale; 2: Unité de Biologie Moléculaire du Gène, INSTUT PASTEUR, 75724 PARIS CEDEX 15, France

1. INTRODUCTION

It has been previously shown that the frequency of cointegration into cellular DNA of two genes borne on the same plasmid molecule varies considerably from cell line to cell line (Colbere-Garapin et al. 1985 & 1986). In monkey kidney Vero cells, cointegration of the entire human hepatitis B surface (HBs) antigen (Ag) gene and the aminoglycoside 3'-phosphotransferase (APH3') gene occurs only in 15 % of the transformed clones whereas in murine LM cells under the same conditions, both genes are cointegrated into 100 % of the clones (Colbere-Garapin et al. 1985 & 1986). In Vero cells, DNA rearrangements and deletions in the exogenous DNA could occur at any one of the 3 stages of the transformation process: 1 - during the first days after transfection by degradation of free plasmid DNA molecules, 2 - during recombination with cell DNA and/or the early replication cycles following integration or 3 - later after integration if the transgenome is unstable. We therefore investigated the penetration, persistence and integration of cotransferred genes carried by double-stranded (ds) DNA molecules in monkey kidney Vero cells and 3 cell lines of human origin, HeLa, GM4312A and HepG2, in comparison with similarly transfected murine LM cells. Furthermore, we have compared the expression of transfected genes borne on single-stranded (ss) and ds DNA molecules in order to find out whether transient gene expression could be obtained from ss DNA, and whether stable coexpression of two genes could occur if distinct ss DNA molecules carrying each of the genes were co-transferred.

2. MATERIALS AND METHODS

2.1. Enzymes, bacterial strains, plasmids and phage. Bacteriophage T4 DNA ligase and restriction endonucleases were purchased from New England Biolabs, Appligene and Boehringer. Escherichia coli strain 803 su III rK m8 used for transformation was kindly provided by K. Murray. Construction of plasmids pAG60 (Colbère-Garapin et al. 1981), pAG400-2 (Colbère-Garapin et al. 1985), pAG452-2 and pAG480-15 (Colbère-Garapin et al. 1986) has been described. BMK 71-18 [Δ (lac-pro AB), thi, sup E ; F'lac i 9, ZMc15, pro A^{+} A^{+}] (source: B. Müller-Hill) was kindly supplied by Dr. H.J. Fritz. Phages pAG511-2 and pAG511-6 are derivatives of M13 mp19 (Messing et al. 1977) into which the HBs gene was inserted. The replicative form of M13 mp19 (Kramer et al. 1984) was linearized with Bam HI and ligated to a 2400 bp Bgl II DNA fragment carrying the HBs gene with two HBs promoters in tandem. The recombinant phage pAG511-2 carries the HBs gene such that it is transcribed into functional HBs mRNA, while pAG511-6 only encodes if transcribed an anti-sense HBs RNA. The hygromy-
cin B (Hy) phosphotransferase gene (HPH) under the control of the herpesvirus hominis 1 thymidine kinase promoter confers resistance to Hy to cells. This hybrid gene, carried by a 1940 bp Pru II fragment, was inserted into M13 mp19 at the Sma I site of the polylinker. Two recombinant phages were obtained: pAG510-17 and pAG510-14. The former encodes HPH mRNA and the latter, if transcribed, encodes an anti-sense HPH RNA. Single-stranded DNA was purified on benzoylated naphthoylated DEAE-cellulose (Gamper et al. 1985).

2.2. Eukaryotic cell and cell transformation. HeLa, TK- mouse LM cells clone ID and monkey kidney Vero cells clone VC10 were cultivated in DMEM supplemented with 10% calf serum. Human hepatoma Hep G2 cells (Aden et al. 1979) were cultivated in MEM containing 10% fetal calf serum (FCS). GM4312 A cells were obtained from the NIGMS Human Genetic Mutant Cell Repository and are derived from human Xeroderma pigmentosum cells transformed by simian virus 40 (SV40). They were cultivated in DMEM supplemented with 10% FCS. Cell transformation with plasmid DNA was carried out as described (Colbère-Garapin et al. 1985) using the calcium precipitation technique. Twenty-four hours after transfection, cell culture medium containing G418 (Gibco) or Hy (Eli Lilly) was added to the cells. The final concentration used was 150 μg G418/ml for Vero, HeLa and LM cells, 100 μg G418/ml for GM4312 A cells, 300 μg G418/ml for Hep G2 cells, and the hygromycin B concentration used was 150 μg Hy/ml.

2.3. Isolation of plasmid DNA after transfection. Between 24 hr and 3 weeks after transfection, plasmid DNA was isolated from the cells by the method of Hirt (1967). High molecular weight DNA was precipitated in 1 M NaCl and pelleted. Plasmid DNA which remained in solution was treated with ribonuclease from bovine pancreas (Calbiochem) at a concentration of 10 μg/ml for 30 min at 37°C. Proteinase K (Merck) was then added at a concentration of 200 μg/ml and the extract was further incubated at 50°C for 30 min. After a phenol-chloroform isoamyl alcohol (24-24-1) extraction, plasmid DNA was precipitated with ethanol.

2.4. Analysis of DNA from transformed cells. Cellular DNA was extracted as previously described (Colbère-Garapin et al. 1981). After cleavage with restriction enzymes, DNA fragments were analyzed according to Southern (1975). DNA fragments used as probes specific for the Ap, Tc and HBs genes were isolated as described (Colbère-Garapin et al. 1986) and 32P-labeled by nick-translation (specific 32P activity 3 x 108 cpm/μg). A Bam HI-Bam HT DNA fragment isolated from plasmid pLG89 (Gritz and Davies, 1983) was used as a probe specific for the HPH gene. Filters were hybridized at 68°C and autoradiographed. Some autoradiographs were scanned with a Gelman gel scanner.

2.5. Detection of the HBs antigen. HBsAg detection in cell supernatants was performed by solid phase radioimmunoassay using an Ausria II kit (Abbott Laboratories).

3. RESULTS

3.1. Penetration and persistence of free double-stranded plasmid molecules in monkey kidney Vero and mouse LM cells. We compared the efficiency of plasmid penetration and persistence in monkey Vero and mouse LM cells. Twenty-four hours after transfection with pAG60 carrying the APH3' selective marker or pAG452-2 carrying the hygromycin B resistance gene, low molecular weight plasmid DNA was extracted from the transfected cells by the method of Hirt (1967) and analyzed by Southern blotting using a