ISSN 0022—8769 Juntendo igaku
CODEN : JUIZAG 32 (4)405～556 (1986)

順天堂医学

JUNTENDO MEDICAL JOURNAL

第32巻4号 （通刊724） 1986年12月

順 天 堂 医 学 会

特　集　分子免疫学の最近の進歩

トランスフェクションを用いた遺伝子発現機構の解析**

DNA Transfection : Gene Regulation, Gene Amplification and Gene Cloning

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遺伝子トランスフェクションの効率を高めるため，筆者らが考案したいくつかの方法を紹介する．また，FACSを用い遺伝子のアンプリフィケーションの細胞生物学的機構を解析した．

索引用語：DNAトランスフェクション，FACS，遺伝子アンプリフィケーション，遺伝子クローニング

Key words : DNA transfection, FACS, gene amplification, gene cloning

Introduction

DNA transfection is a powerful tool of gene analysis. Indeed it allows functional studies of genes either from high molecular weight cellular DNA (i. e. in their physiological structure) or cloned in vectors (i. e. amenable to manipulations designed to influence their function). The work described here shows how this technique has been applied to the problem of DNA methylation and gene regulation, to the study of the amplification of surface antigen genes and to gene cloning.

Material and Methods

Cell lines : The human choriocarcinoma cell line Jar was cultured in Waymouth's medium. Thymidine kinase$^-$L cells were cultured in DMEM.

DNA : Most cellular DNA were prepared using the method of lysis by guanidinium thiocyanate followed by cesium chloride banding[1]. Plasmid DNA was prepared by conventional techniques of centrifugation in ehidium bromide／cesium chloride gradients.

DNA transfection : Cotransfection by the calcium phosphate precipitation technique was followed[2].

Immunofluorescence : Fluorescence analyses and sorts were performed on a FACS II essentially as described[2].

Results and Discussion

DNA methylation : Trophoblast cells are among the few nucleated cells in the body that do not express HLA class I antigens. A very tight mechanism of regulation is likely to be responsible of this lack of expression. Since DNA methylation is a covalent modification of the DNA and seems to be a mechanism that cells use to turn off genes irreversibly, we hypothesized that it would prevent the expression of

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**第238回順天堂医学会学術集会〔*Sept.* 18, 1986〕
(Oct. 30, 1986受付)

Table 1 Transfection efficiency of various sources of DNA

	Jar		Aza-C*		Leukocytes
	(untreated)	12days	6~8weeks	4~6months	
HLA	0/16**	4/10	6/13	0/14	6/7
Leu-2	0/16	4/11	1/1	0/13	15/25

* : DNA from Jar cells treated with Aza-C : time after the treatment.

* * : Number positive/number transfected dishes.

these genes after transfection. The transfectability of HLA class I and of a T cell differentiation antigen (Leu-2) was investigated using DNA from choriocarcinoma cell lines (tumors of trophoblastic origin). DNA from Jar cells did not transfect the antigens studied (Table 1) whereas all the markers were tronsfected using leukocyte DNA. Intermediate results were obtained using DNA from other choriocarcinoma cell lines (data not shown). Jar cells were treated with azacytidine (Aza-C) to decrease the level of methylation of their DNA. Since culture of the treated cells allows remethylation to occur, DNA were extracted at various time points after the treatment and transfections followed by FACS selection were performed. As shown in Table 1, DNA from Jar cells treated with Aza-C becomes able to transfect HLA class I and Leu-2, but after a period of time in culture it can no longer transfect for these markers. Transfection efficiencies correlate fairly well with the general degree of methylation of the genes. These results indicate that methylation can prevent the expression of genes after transfection and support the idea that this is an important physiological mechanism to prevent expression of these genes in the trophoblast.

Gene amplification : Increase in the number of copies of various genes has been observed in many different kinds of cells including tumors and cell lines in culture. Gene amplification has also been observed in L cells transfected for the Leu-2 gene[2]. The availability of a simplified model of DNA amplification, like the one offered by DNA transfection, would greatly facilitate the study of the basic mechanisms of this phenomenon. Thus we decided to investigate if amplification of transfected surface antigen genes is of common occurrence. L cells expressing the antigens Leu-1, Leu-2, Trop-1 and Trop-2 were selected. The brightest cells of independent transfectants were cloned and the expression of the transfected markers was studied (Table 2). It was observed a high frequency of increased expression for Leu-1 and Leu-2 ; the frequency was lower for Trop-2 and only one example of increased expression was found for Trop-1. The analysis of the number of copies of the transfected genes is ongoing. However, it is clear that, although increase in expression is possible for all the genes studied, the frequencies are different, possibly reflecting the presence of structural differences among the various genes.

Table 2 Levels of expression of transfected surface antigens.

	relative amounts of surface antigens*		
	unchanged	twofold increase	more than twofold
Leu-1	2/10**	4/10	4/10
Leu-2	2/13	3/13	8/13
Trop-1	6/7	0/7	1/7
Trop-2	3/6	0/6	3/6

* : The comparison is relative to the cells the clones were derived from.

* * : Number of analyzed/total number of transfectants.

Gene cloning : The rationale of our approach is the following. A vector containing sequences of the human BK virus has been shown to replicate as a stable episome in human cells[3]. A genomic library will be constructed in a modified version of this vector and will be transfected in human cells. After selection of cells expressing the gene of interest (for example FACS selection of surface antigen genes) a simple Hirt extraction will allow the isolation of the episomal DNA, thus virtually completing the process of cloning. The characteristics of the vector and the influence of different selectable markers on the efficiency of expression of genes subcloned into the vector will be discussed.

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