TRANSFECTION AND CLONING OF GENES FOR MEMBRANE ANTIGENS USING THE FACS

LEONARD A. HERZENBERG*, CHARLES HSU, SAVERIO ALBERTI and PAULA KAVATHAS
Department of Genetics, Stanford University, Stanford CA 94305, U.S.A.

(Received 7 June 1984)

In order to facilitate cloning of genes for cell surface molecules, we cotransfected LTK- mouse fibroblasts with thymidine kinase (TK) genes and total human or mouse DNA. TK + cells, selected by growth in HAT medium, were stained with fluorochrome conjugated monoclonal antibodies or other fluorescent ligands which bind to one or another membrane differentiation antigen or receptor. We isolated fluorescent transfectants expressing these molecules using a fluorescence activated cell sorter (FACS). For some antigens, spontaneous gene amplification occurred. By repeated cycles of FACS sorting and regrowth we obtained high expressing clones. We then isolated cDNA and genomic clones using selected cDNA probes to screen phage with cDNA inserts. DNA from virtually any tissue source transfected equally well for the various molecules except for DNA from a trophoblast derived choriocarcinoma cell line which did not transfect for Leu-2.

Key words: Cotransfection, FACS, Gene amplification, Leu-2, Differentiation antigens.

INTRODUCTION

The development of the whole field of immuno-flow-cytometry has depended upon the existence of specific glycoprotein cell surface molecules which are sufficiently different antigenically so that monoclonal antibodies can be made which react with certain marker molecules on one functional type of cell and with other such molecules on another functional cell type. The fortunate development of hybridoma technology for generating monoclonal antibodies,1 coming just as the development of fluorescence activated cell sorter or flow fluorescence technology was reaching a high level of technical reliability, allowed this field to flourish. (For a recent review, see Ref 2.) Although some of the antigens detected by these antibodies have been studied biochemically, very little is known about their primary (amino acid sequence) structure. This is because, with very few exceptions, such as membrane immunoglobulins and products of the MHC loci, they are present on the surface in rather small amounts and usually turn over metabolically quite slowly.

Despite this, we and others have postulated homologies between molecules found on the surfaces of human cells and murine cells.3 For example, we have proposed the following homologies based in part on their distribution on functional cell types: (1) the Lyt-2,3 molecule, which marks suppressor and cytotoxic cells in the mouse, is homologous to the human Leu-2 (T8) molecule, which marks suppressor and cytotoxic cells in the human; (2) Ly-1, which is present on all T-cells in the mouse, albeit in higher density on helper cells than on suppressor or cytotoxic cells, and which is found on a subpopulation of B-cells,4 is homologous to the Leu-1 (T1) molecule. Homologies have also been proposed for murine L3T4 and human Leu-3 (T4),5 and for Leu-4 (T3), which is associated with the T-cell antigen receptor molecule, in the human, and an unnamed molecule in the mouse associated with the murine T-cell receptor.6

In addition to identifying these molecules by their distributions on functional cell types, studies of the polypeptide chain compositions and approximate mol. wts have supported the homology assignments that we made.3 Nevertheless, a geneticist’s definition of homology is based upon evolutionary relationships, most easily defined by amino acid sequence and nucleic acid sequence comparisons. To facilitate sequence studies on these molecules, we have embarked on a program of isolating and cloning the genes which code for these molecules since sequencing can be much less laborious at the nucleic acid level than at the protein level.
COTRANSFECTION APPROACH TO CLONING

Since these molecules are not very abundant and turn over relatively slowly, we expect the level of corresponding mRNA also to be rather low. Thus, we decided to use an approach to gene cloning which did not depend upon isolating enough antigen to determine an amino acid sequence and using this sequence to make corresponding nucleic acid probes for screening a library. Although this is a powerful technique for gene cloning, we were afraid it would be too difficult to use for many of the cell surface antigens we were interested in. Instead, we decided to use another approach, one that has proved to be successful for isolating genes coding for housekeeping enzymes and a number of oncogenes. This is the approach of DNA mediated gene transfer or DNA transformation or transfection. 7 In our case, the plan was to cotransfect total cellular DNA with a suitable selectable marker, thymidine kinase (TK), using a good recipient cell line, LTK- mouse transformed fibroblasts deficient in this enzyme. After selection for TK+ transfectants in HAT medium, we would stain with one or another monoclonal antibody or ligand coupled to a fluorochrome and use the fluorescence activated cell sorter (FACS) to isolate specific cell surface molecule transfectants. Then we expected that one or another kind of 'marker rescue' method could be used to isolate the gene coding for the transferred antigen. As it turned out, our original idea was a good one in that it led to our being able to clone the gene for one cell surface molecule and being well advanced in efforts to clone several other molecules using the transfection approach. However, the details of how we isolated and cloned the genes had to be changed in the light of actual experimentation. This is an example of where the idea was good, not because it was totally correct, but because it suggested useful experiments, which, helped by the hand of serendipity, led to a successful outcome.

SELECTION OF TRANSFECTANTS

In Fig. 1, we show the general scheme for isolation of transfectants. 8 DNA extracted from almost any human or murine tissue or cell line mixed with chicken or herpes simplex virus TK is precipitated with calcium phosphate and sprinkled on monolayers of LTK- cells. After 48 h, the cells are washed and HAT medium added. After 2 weeks incubation, we have about 1000 TK+ colonies per million cells plated. These cells are suspended, mixed with fluorochrome conjugated monoclonal antibodies or ligands (or in some cases with unconjugated antibodies or ligands followed by fluorochrome conjugated second step reagents) and analyzed and sorted using the FACS. Positive cells are enriched and finally cloned using the FACS. The lists in Table 1 show that we successfully transfected for a wide variety of human and mouse cell surface molecules using this technique. The only limitation seems to be that transfection for only one gene at a time is required. The frequency of antigen transfectants is in the range of 1 per 1000 among TK+ cells. Thus, if two genes have to be simultaneously transfected to produce a surface molecule, we expect to have only one stained cell per million TK+ cells or per 1012 originally treated cells. This is too large a number of cells to conveniently try to transfect. Nevertheless, we have successfully transfected for some two chain