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Transfection as an Approach to Understanding Membrane Glycoproteins*

A. Fortunato, R. F. L. James, A. Mellor, and N. A. Mitchison

Gene transfection has much to contribute to our understanding of membrane glycoproteins. The technique is in principle simple: it consists of the transfer of a single gene from one cell to another, using the method of DNA recombination in plasmids to manipulate the gene during the transfer and to rescue it for analysis afterwards. This is valuable for several reasons. The first and simplest is that it generates a cell which has a single new gene product. As the functions of most gene products are still unknown, this should greatly help us to find out what these functions are. For example, the function of the great majority of membrane glycoproteins such as Thy 1, Lyt 1, and T5/T8 remains to be understood. Most membrane glycoproteins have so far been defined only as antigens, sometimes and to an increasing extent through the use of monoclonal antibodies. It turns out to be very difficult to find out what these glycoproteins do, even after quite a lot has been found out about their structure. The Thy 1 molecule is a case in point. It was discovered 18 years ago, it has been used as a marker in lymphocyte differentiation for 13 years, and its primary structure has now been unravelled [23], yet we still know next to nothing about its function. Up to a point the classical approaches of genetics can be applied to these problems: analysis by means of loss and temperature-sensitive mutations. Nowadays these may be supplemented by segregation analysis, in which a cell positive for a given glycoprotein and a given function is fused with a negative cell and the daughter cells analysed for co-expression of the glycoprotein and the function [3]. But progress using classical genetics has been slow, and the contribution to be exprected from transfection is accordingly great. With some justice one could argue that transfection is not a new departure in principle since it merely uses positive variants where classical genetics uses negative variants. However there are many reasons for expecting these positive variants to be far more valuable.

While it is the natural functions of membrane glycoproteins which are likely to interest us most, there are other functions – sometimes termed "pseudofunctions" – which are well worth attention. Activity as an antigen is a good example. Mammalian membrane glycoproteins generally function as antigens only in the course of clinical procedures such as blood transfusion and organ transplantation, or in experiments. Yet this activity is of great interest, not only because of its importance in influencing the outcome of clinical procedures, but also because of the information which allo-immunization provides about the working of the immune system [14]. This is one of the areas in which transfection is likely to have a major impact.

Apart from its application in understanding the function and pseudofunction of gene products, transfection is of importance in formal and reversed genetics. In formal genetics transfection can be used as a starting point for examining gene structure, and for locating and enumerating genes within the genome. Its use for these purposes is

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complementary and to some extent competitive with biochemical approaches to gene cloning. It provides a method of constructing DNA probes which is alternative to the standard route of isolating specific mRNA and proceeding via cDNA. In reversed genetics – still largely an undeveloped subject – it is envisaged that genes will be varied structurally prior to transfection. This will generate altered products of structural genes, primarily for use in structure-function analysis. It will also generate genes with altered control sequences, for use in analysis of the control of transcription.

As a technique for analysing the function of glycoproteins transfection does not stand alone. Perhaps its most formidable competitor is the use of liposomes. Individual glycoproteins can be isolated by means of standard methods in protein chemistry, and then incorporated into liposomes for functional testing. This approach is being followed, for example, in studies of cytotoxicity mediated by T cells [8]. It is yielding results of interest, and there is scope for considerable extension of the approach if and when genetically engineered glycoproteins can be produced in bulk. At present it has the disadvantage that only very limited quantities of the glycoproteins in interest can be produced, and there are problems about purity.

A. Transfection in Immunology

We present a list of the future applications of transfection in cellular immunology which seem to us particularly urgent. Obviously any such list is arbitrary and incomplete, and reflects our individual interests. Nevertheless we hope that it may be useful in provoking further thought by ourselves and others.

I. Unification of Function

Cell surface markers have often attracted interest from several points of view, and have accordingly been studied by means of several different assays. In such cases there may be doubt whether one is dealing with one or several distinct membrane molecules. A case in point is the major histocompatibility complex, where products of the class II region have been studied as antibody-defined alloantigens, as lymphocyte-defined alloantigens, as "restriction elements" or guides for self-lymphocytes, and as controllers of immune responsiveness. Another is H-Y, which has been studied as an alloantigen defined by cytotoxic T cells, helper T cells, suppressor T cells and antibodies, and also as a controller of sexual differentiation [22]. In the case of the MHC it is argued that all these functions are exercised by each of the members of a small family of glycoproteins, which probably number no more than two for the class II molecules of the mouse [9]. This view, the "unified" view of the MHC, rests on evidence from two sources: analysis of spontaneously occurring mutations and the use of monoclonal antibodies. It is likely that transfection experiments will further document this unification, and indeed this process has already begun [12]. It will be more interesting to see what happens in the case of H-Y, where the outcome is more in question.

II. Alloantigens: The Ultimate Congenic Lines

In the past alloantigens have been defined and studied through the use of congenic strains and recombinants between congenic strains. Most of this work has been done in the mouse, where the production of congenic strains and their recombinants has become a major and very expensive international industry. This work is being duplicated in other even more expensive species, such as the rat and the chicken. In the chicken, because of the shorter genetic length of the MHC, ten times as many animals will be needed in each informative cross [18] (unfortunately cell lines are hard to produce in chicken, and no transfection-susceptible cell is yet available). At the end of all this effort is still uncertainty about functions defined in this way, mainly because of possible contamination of the desired gene by flanking genetic material. In a recent study, for example, Dresser used an immunoglobulin allotype which had been backcrossed for 20 generations only to find that his results could be attributed to

genetic contamination. This he was able elegantly to identify as a minor transplantation alloantigen [7].

In our own work on alloantigens recognized by regulatory T cells the problem of genetic contamination has been a recurring theme. Thus some years ago we defined a population of helper T cells which appeared to recognize the class I MHC molecular H-2K, but we could not exclude the possibility that their true target was an unidentified class II molecule [17]. More recently we encountered the same kind of problem with a helper cell line recognizing an unexpected H-2A specificity [24]. Looking outside the MHC, we have had problems with Thy 1. On the whole the evidence suggests that T cells do not recognize Thy 1, and in a primary response system colleagues in this laboratory have shown that helper T cells definitely do not do so [11]. Nevertheless hyperimmunization appears to generate a population of helper cells which do recognize the molecule, if we assume that our Thy 1 congenic strains are truly congenic [4]. Obviously this assumption is open to question.

III. Transfection-Generated Congenics and the Genetics of Immunological Responsiveness

The genetic control of immunological responsiveness to alloantigens has become an active subject. Several careful studies of responsiveness to MHC molecules are under way elsewhere [5] and colleagues in this laboratory and elsewhere have tried to analyse responsiveness to Thy 1 [6, 25]. The work is going slowly because it is limited by the availability of congenic strains. There are few instances where an alloantigen is available on a medium range of paired congenic strains, and none except H-Y where such pairs are really widely distributed. That is one reason why the genetics of responsiveness to H-Y has attracted so much interest [19, 21], and why it has led to fundamental advances in our understanding of this whole subject (for an example of this see our account of the work of M. Brenan [15]). Transfection could greatly broaden the material suited to this kind of analysis.

IV. Characterization of Minor Alloantigens

H-Y has already been mentioned as an alloantigen defined by T cells, and it was in fact discovered in this way. The same is true of most other minor alloantigens, and indeed of some medial alloantigens as well. Nearly all these minor antigens can at present be defined only by means of T-cell responses [1]. The same applies to tumour-specific transplantation antigens, including not only the classical antigens of chemically induced tumours in mice, but also the very interesting antigens defined recently on UV-induced tumours [10] and mutagenized "tum" variants [2]. These UV tumour antigens are arguably the counterpart in mice of the only antigens known to mediate immune surveillance in man [16]. We have long been interested in murine minor alloantigens because of the range of reactivity with regulatory T cells which they offer. One may rationally hope that this range of reactivity will eventually generate rules about how to construct a helper or suppressor epitope [13]. But we cannot properly exploit all the immunological information without having also the relevant structural information. And for T-cell defined antigens this information has long seemed inaccessible. All this has now been changed by the advent of transfection. In fact we can now expect to have DNA sequence information about minor antigens long before we have protein chemistry.

V.

We close this list simply by mentioning two molecules central to modern immunology, IgD and I-J, one of which has a structure without a function and the other a function without a structure. Both of these enigmas we believe will be resolved by means of transfection.

B. Recent Work on Transfection

We, in collaboration with colleagues elsewhere, have been transfecting mouse genes encoding membrane glycoproteins belonging in class I of the MHC [12]. Several groups have already isolated cDNA and

genomic DNA clones containing class I MHC genes of the mouse (cited in [12]). In order to screen our cosmid library we used cDNA probes obtained from elsewhere. We did in fact carry out some initial work on screening directly by expression, and we believe that in the long run this may prove a viable strategy for screening genomic clones for other glycoproteins such as Thy 1. Detailed information about the preparation and screening of the cosmids is presented elsewhere in this volume by E. Weiss, and we present here further characterization of the transfected cells. In the initial report we described a radioimmunoassay which detected expression of transfected H-2K^b and H-2D^b genes by means of monoclonal antibodies. We here present fluorescence-activated cell sorter analysis of these transfected cells using the same monoclonals.

Table 1. Control titration of anti-H-2K^b (Y25) and anti-H-2D^b (B22.249.RI) monoclonal antibodies on L cells (C3H) and EL4 cells (B10)

Antibody titration	Cells used[a]	
	EL4	LDI
NMS 25[b]	440± 126	521± 63
Y25 (anti-K^b)		
2×10^{-3}	8,650± 917	637±123
6×10^{-3}	7,620± 805	641± 49
18×10^{-3}	6,848±1,246	NT
54×10^{-3}	3,461± 834	NT
162×10^{-3}	1,344± 412	NT
B22 (anti-D^b)		
40	15,355± 353	574±129
120	7,736± 335	483± 67
360	2,776± 158	NT
1,080	1,080± 52	NT
3,240	595± 16	NT

The monoclonal antibodies used for screening transfected LD1 cells were titrated by a radiobinding assay utilizing affinity-purified ^{125}I-rabbit anti-MIg as the second label. EL4 cells (H-2^b) were used as the positive control. Antibodies used were Y25 (anti-H-2K^b) (kindly provided by Dr. E. A. Lerner and B22.249 RI (anti-H-2D^b) (kindly provided by Dr. L. Herzenberg)

[a] Results expressed as ^{125}-I-rabbit anti-MIg c.p.m. bound ± S.D. (total counts applied = 100,000)

[b] Titration expressed as the reciprocal of the antiserum dilution used

Table 2. Fluorescent activated cell sorter analysis of mouse L cells (H-2^k) transfected with H-2K^b and H-2D^b genes

Cells	Antisera			
	NMS 1/100	Y25 (αK^b) 1/1000	B22 (αD^b) 1/100	αH-2^k 1/100
LDI	7	7	7	99
EL4	7	96	79	13
LH8	8	40	7	NT
LH8.1	6	94	11	98
LH8.2	16	98	NT	NT
LB3.2G	16	9	54	100
LB1.1.1	7	NT	50	95

Results are expressed as the percent positive cells over background level (cells plus FITC-anti-MIg). FACS analysis of transfected LD1 cells are expressed as the percent positive cells (gates 41 – 255) over the background level (set at gates 1 – 40). Antisera used: NMS 1/100, normal mouse serum; Y25 1/1000, monoclonal anti-H-2K^b); B22 1/100, monoclonal anti-H-2D^b; B10 αC3H, 1/100, allo-antiserum (absorbed EL4 cells). Cells used: LD1, Tk$^-$ mouse L cells (H- 2^k); EL4, mouse T-cell lymphoma (H-2^b); LH8, LD1 cells transformed with H-2K^b gene (uncloned); LH8.1/LH8.2, LD1 cells transformed with H-2K^b gene (cloned); LB3.2G/LB1.1.1, LD1 cells transformed with H-2D^b gene (uncloned)

Control data showing the expression of K^b and D^b on B10 (H-2^b) cells (EL4) and the lack of expression on C3H (H-2^k) cells (L) are presented in Table 1. The same monoclonals were then employed for FACS analysis shown in Fig. 1. In this analysis the cloned cell line LH 8.1 (H-2^k transfected with K^b) shows as essentially 100% K^{b+} and 0% D^{b+}, while the as yet cell line LB3.2G (H-2^k transfected with D^b) shows as essentially 0% K^{b+}, and with a major fraction of cells D^{b+}. The corresponding numerical data from this FACS analysis are presented in Table 2.

What these data establish is that transfected gene products can successfully be picked up on FACS analysis. This is a step forward towards screening by expression,

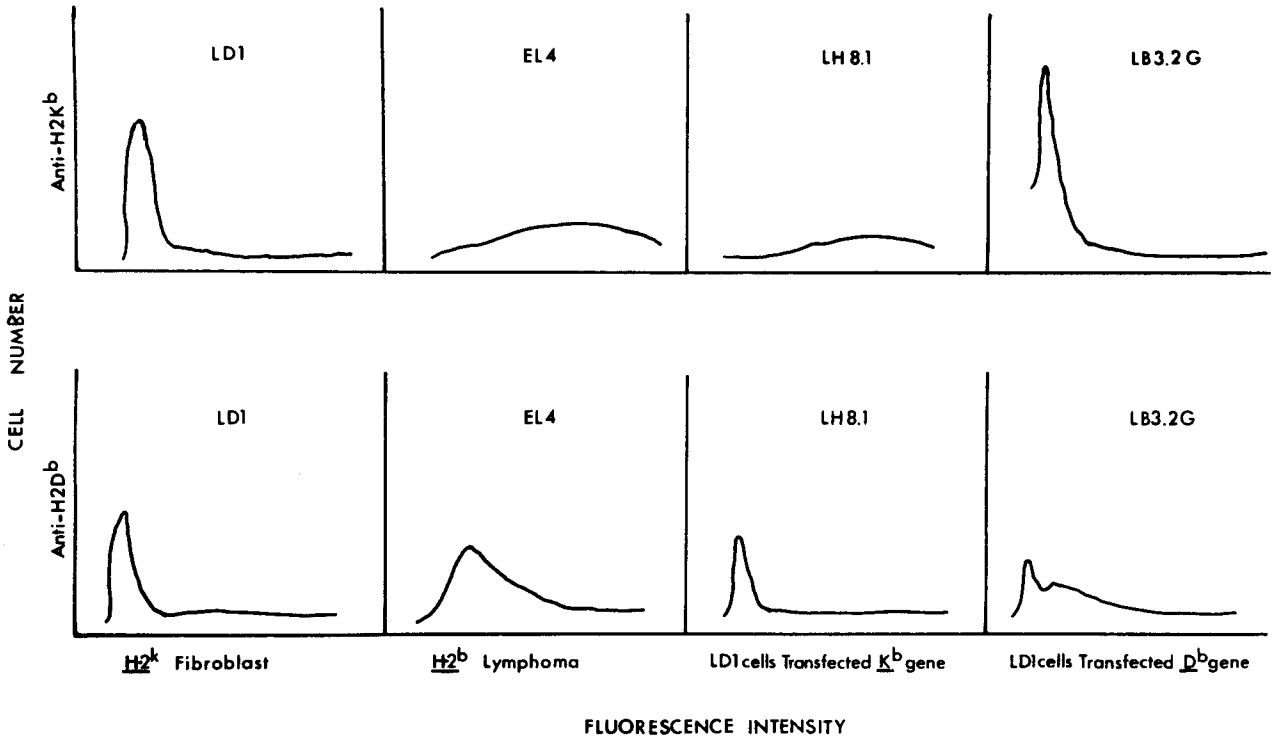

Fig. 1. Fluorescence-activated cell sorting (FACS) using the Beckton-Dickinson FACS IV. For each experiment the laser power was set at 200 mW, the photomultiplier at 730 V, the scatter gain at 4/0.8 and the fluorescent gain at 16/0.8. Generally cells falling between gates 1–40 were considered negative while those falling between gates 41–255 were positive. A total of 10^4 cells were considered in each profile. Cells used: LD1, Tk^- mouse L cells (H-2^k); EL4, mouse T-cell lymphoma (H-2^b); LH 8.1, LD1 cells transformed with H-2K^b gene (cloned line); LB 3.2G, LD1 cells transformed with H-2D^b gene (uncloned line). Monoclonal antibodies used: Y25, anti-H-2K^b (1/1000); B22.249.RI, anti-H-2D^b (1/100)

where it is expected that FACS analysis will be used to clone cells positive for expression.

C. Further Prospects

The recipient cells used in this work are L cells, which unfortunately have lost Thy 1 expression. Using a sensitive immunization assay we have confirmed a previous report to this effect based on immunofluorescence [20]. Our immediate aim is to study regulatory T cells directed at the products of transfected genes, and for this purpose it is almost essential to use Thy 1 as a read out antigen detected by B cells. Accordingly, we are now engaged in transfecting rat cells known to be positive for Thy 1.1. We are also trying to isolate genomic clones encoding Thy 1; it will not have escaped general attention that the published amino acid sequence contains at least a portion attractive for its low ambiguity [23]. In the future the whole programme outlined in our introductory section lies open.

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