REVIEW ARTICLE

Cell-surface-antigen mutants of haematopoietic cells

Tools to study differentiation, biosynthesis and function

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Rationale for and limitations of a somatic genetic approach for studying cell-surface-antigen biosynthesis and function

Serological heterogeneity of haematopoietic cells

One of the major concepts of developmental biology is that diverse cell lineages arise from a common progenitor cell. Important among the objectives of developmental biology is to understand the lineage relationships of those cells that descend from a single progenitor, to understand the events that lead to commitment of a progenitor cell to a specific developmental pathway, and to understand how the steps of differentiation along a particular pathway are regulated.

The haematopoietic system has been one of the most accessible systems for studying these questions (Till & McCulloch, 1980). Physiological assays (Till & McCulloch, 1961; Wu *et al.*, 1968; Metcalf, 1977; Abramson *et al.*, 1977) have established that the various cells of the haematopoietic system are the descendants of progenitor cells of restricted developmental potential that descend from a common pluripotent stem cell. How the progeny of an individual stem cell become committed to one or another lineage is still a matter of controversy (Till & McCulloch, 1980; Humphries *et al.*, 1981; Nicola & Johnson, 1982). However, it seems reasonable to assume that the differentiation of a progenitor along a particular pathway is a regulated process and involves the interaction of progenitor cells with specific regulatory molecules.

On this basis, cell-surface molecules, which may show differential expression on particular subpopulations of cells, become of considerable interest since it is a reasonable hypothesis that at least some of these molecules are receptors for ligands that act to regulate cell growth and differentiation. Alternatively, molecules that show differential expression may be involved in functions that require the cell to interact with its environment, functions such as homing to particu-

lar locations or, as in the case of the cells of the immune system, recognizing and responding to foreign substances.

The initial evidence that cell-surface molecules are differentially expressed on certain haematopoietic subsets came from serological studies on lymphoid cells (Boyse & Old, 1969; McKenzie & Potter, 1979). The use of monoclonal antibodies (Williams *et al.*, 1977) has permitted a much more extensive search for such molecules. The general conclusion that has emerged is that molecules (or, really, antigens) that show qualitative or quantitative differences in cell-surface expression on different populations within the haematopoietic system do occur (McKenzie & Potter, 1979). However, rationalizing the distributions that have been seen (see Milstein & Lennox, 1980) and ascribing a function to any particular molecule have been more of a problem.

Use of tissue culture cell lines to overcome the heterogeneity of normal cell populations: advantages and limitations

To study the regulation of surface antigen expression with normal haematopoietic cells is difficult, as the cellular composition of haematopoietic tissues is quite heterogeneous and progenitor cells are present in very low frequency. Some progress has been made in purifying progenitors from normal populations (Nicola *et al.*, 1981; Watt *et al.*, 1983; Nijhof & Wierenga, 1983) and in growing these cells *in vitro* (Metcalf, 1977; Dexter & Testa, 1980; Nicola *et al.*, 1981; Humphries *et al.*, 1981; Nakahata & Ogawa, 1982) but it is still hard to obtain pure populations of continuously proliferating cells in quantities suitable for genetic and molecular analysis.

An alternative is to use cell lines adapted to tissue culture as representatives of normal cells. Cell lines have the advantage that they provide homogeneous populations of cells in quantity. Therefore, many of the techniques of genetics and molecular biology can be used to analyse the biosynthesis, regulation and the biological function of cell-surface molecules of interest.

Abbreviations used: MHC, major histocompatibility complex; HLA, human leucocyte antigen; P, phosphate.

One approach is to use antibodies that define molecules that show differential expression to select for cell-surface-antigen mutants. The use of cultured cells permits selection for mutations that might be lethal in the intact animal. Genome interactions can be studied at the cellular level by studying antigen expression in hybrids between cells in different states of differentiation. For an increasing number of cases, regulation can also be studied by transfection of normal or mutant genes into appropriate recipient cell lines. Cell lines that differentiate *in vitro*, in response to inducing compounds (Ichikawa *et al.*, 1976; Marks & Rifkind, 1978; Maki *et al.*, 1980), can be used to study the regulation and significance of changes in the expression of cell-surface molecules during differentiation. Finally, surface-antigen mutants of cell lines that are dependent for growth on specific factors (Dexter *et al.*, 1980; Greenberger *et al.*, 1983) or that express assayable functions can be used to study the relationship between a specific molecule and the biological function of the cell.

Although all these points are advantages, the limitations of using cell lines should be understood. Cells in culture and the tumours from which many of these lines have been derived may undergo chromosomal duplications, deletions or rearrangements (Ohno, 1971; Klein, 1979; Siminovitch, 1979; Miller & Miller, 1983). Chromosome loss and rearrangement is even more of a problem in cell hybrids (Worton *et al.*, 1977; Miller & Miller, 1983) so that, at the minimum, studies on a number of independent hybrids must be carried out for any meaningful conclusions to be drawn from hybridization experiments. The isolation of a mutant cell line that shows aberrant expression of an antigen on the cell surface, or the demonstration that a somatic cell hybrid either fails to express a surface antigen or shows expression of a surface antigen not expressed by the two parental lines, is not evidence that the particular regulatory mechanism defined by the mutant or hybrid operates normally. The analysis indicates potential points at which regulation can operate and defines those biosynthetic steps that are essential for expression of a particular molecule on the cell surface, but does not imply that these are points at which regulation occurs during normal differentiation. This is a serious problem, but at this time there does not seem to be any simple way to relate the potential points at which expression can be regulated, as defined in cultured cells, to the actual points at which regulation occurs in normal cells. The information gained from cell lines does, however, indicate where one should look when studying regulation in normal cell populations. Finally, for any given mutant or hybrid an unambiguous mechanistic interpretation can only

be made after a complete analysis *in vitro* has been carried out (Beckwith & Rossow, 1974). This is not always technically possible and in any case requires considerable effort. In its absence, however, molecular interpretations based mainly on genetic analysis must always remain imprecise.

Selection of mutants for cell-surface molecules

Criteria for choosing cell-surface molecules suitable for somatic genetic analysis

Given these limitations, the surface molecule to be studied should be chosen with care. It is essential that a strong, specific antiserum be available against the molecule in question, since without such a serum it is difficult to do clean selections and to carry out serological and biochemical experiments on mutants. From the standpoint of hybrid analysis, sera that detect alloantigenic variation are very useful. Molecular probes are often essential in analysing somatic-cell mutants, and cloned genes permit a wide variety of mutagenesis and transfection experiments to be carried out. Finally, it is intellectually satisfying either if the function of the molecule in question is understood, so that changes in structure can be related to changes in function or, conversely, if the cell line being used has a measurable function so that changes in the expression of particular molecules can be related to changes in cellular function. No one cell-surface molecule totally meets all of these criteria.

Selection of cell-surface-antigen-negative mutants

The first cell-surface-antigen-negative mutants described lacked one haplotype of the murine major histocompatibility complex. These mutants were first obtained by passaging tumours of F₁ animals in parental strains (Bayreuther & Klein, 1958). Selection of mutants in the whole animal, however, is a rather uncontrolled situation. Selection *in vitro* using antibody and complement to kill wild-type cells bearing the antigen in question provides more defined selective conditions. The exact protocol depends on the cell line and antigenic system. Sometimes a single selection with antibody and complement is adequate (Pious *et al.*, 1973; Buxbaum *et al.*, 1977; Hyman & Trowbridge, 1977; Rajan, 1977; Kavathas *et al.*, 1980). For selection of membrane immunoglobulin mutants, however, it was found necessary to carry out two selections within a single cell cycle (Andrews-Wagner & Sibley, 1983). In other cases, such as most Thy-1⁻ mutants (Hyman, 1973), T200⁻ mutants (Hyman & Trowbridge, 1981) and retrovirus glycoprotein mutants (Kabat *et al.*, 1980), several cycles of selection, in which the surviving cells were cultured for days to weeks

after the initial selection and then subjected to further selection, were necessary before mutants were present in sufficient frequency to be isolated. These differences may reflect both the strength of a particular selection (Hyman & Stallings, 1974) and the frequency of pre-existing mutants (or chromosome segregants) in a given cell line.

Antibody-complement selection has the disadvantage that cells expressing low levels of antigen may escape selection (Hyman & Stallings, 1974). Often this technical problem can be overcome by using an anti-immunoglobulin as a second reagent (Hyman & Trowbridge, 1981) or by increasing the concentration of antibody and complement. Several approaches may be used as an alternative to isolate antigen-negative cell lines: selection for fluorescent-negative cells using the cell sorter (Mains & Sibley, 1983) and the use of antibodies conjugated to the toxins ricin (Trowbridge & Domingo, 1982; Mains & Sibley, 1983) and gelonin (Colombatti *et al.*, 1983).

A particularly interesting selective protocol was used for the isolation of mutants for the murine transferrin receptor (Lesley & Schulte, 1984). These workers grew lymphomas in monoclonal antibodies against the murine transferrin receptor. The antibodies blocked cell growth by interfering, in some so far undefined way, with transferrin-receptor function. Selection was for mutants which could escape the growth-inhibitory properties of the antibodies. The mutants obtained, which grew out in the presence of the growth-inhibitory antibody, behaved as structural gene mutants in the gene coding for transferrin receptor. Resistance to antibody was co-dominant in hybrids to wild-type lines, and immunoprecipitation analysis suggested that one copy of the transferrin receptor structural gene in these mutant cell lines was altered while one wild-type allele was retained.

Genetic basis of cell-surface-antigen mutants

Although initially the genetic basis of antigen-negative cell lines was uncertain (Hyman & Stallings, 1974; Rajan & Halay, 1980), there is now direct evidence that these cell lines are true mutants—in the sense of a change in genetic information (Hyman *et al.*, 1980a; Kavathas *et al.*, 1980; Gladstone *et al.*, 1982; Parnes & Seidman, 1982; Orr & DeMars, 1983; Evans *et al.*, 1984). One apparent inconsistency with a genetic explanation is the fact that many mutants that show recessive behaviour in somatic cell hybrids occur in cells that are presumably diploid for the gene in question (Hyman & Stallings, 1974). While the spontaneous mutation rate for loss of one HLA or H-2 allele in a heterozygous line is of the order of 1×10^{-5} – 5×10^{-7} /cell per generation (Pious & Soderland, 1977; Rajan & Halay, 1980), the rate

for loss of Thy-1 in a cell line which is presumably diploid for this gene is of roughly the same order of magnitude (Buxbaum *et al.*, 1977). [Strictly speaking, it has not been shown that this particular Thy-1⁻ defect is due to a mutation in the Thy-1 structural gene; in fact, it is likely that it is in a gene which acts post-translationally (Hyman & Trowbridge, 1978; see below).] The explanation of this similarity in mutation rate is probably that tumour lines show many chromosomal rearrangements, resulting in the frequent segregation of all or a portion of an autosome bearing one wild-type gene (Chasin, 1973). Thus, at any given time a proportion of cells will contain a single copy of the relevant chromosomal region: A fraction of these segregants will contain a mutant allele. This hypothesis is supported by Southern blotting analysis of mutants with defects in the structural genes coding for β_2 -microglobulin (Parnes & Seidman, 1982) and Thy-1 (Evans *et al.*, 1984). In both of these cases the defect is in a gene which is autosomal. In the mutant cell lines a single structurally altered gene is present while the wild-type gene is not present. The considerable difference in frequency of a specific mutation among cell lines (Hyman & Trowbridge, 1978) may thus be explained as due to a markedly different probability that a specific chromosomal region will be lost. In most cases (Hyman & Trowbridge, 1977; Pious & Soderland, 1977), mutagenesis increases the frequency with which antigen-negative cells occur.

The nature of the genetic lesions that result in a mutant phenotype has been easiest to study in selections carried out in heterozygous cells against the genes of the major histocompatibility complex. In this situation nearly all of the mutations appear to occur in the structural genes selected against, the chromosomal location of these structural genes is known, and the genes of the complex are present as a series of closely linked loci. When selection is carried out against one allelic gene product, using polyclonal antibodies in non-mutagenized cells, stable loss mutants are obtained which express none of the specificities that characterize the allelic gene selected against (Rajan & Flores, 1977; Pious *et al.*, 1980). In most cases *cis*-linked unselected markers are still present, arguing that deletions of greater than one centimorgan have not occurred (Pious *et al.*, 1980). Sometimes, however, loss of linked unselected markers can be demonstrated (Rajan & Flores, 1977), suggesting, although not proving, that large deletions may occur spontaneously. Linked marker analysis of mutagenized cells indicates that extensive deletions extending through the major histocompatibility complex and into neighbouring genes may occur, although most lesions are more restricted in extent (Pious *et al.*, 1980; Kavathas *et al.*, 1980; Gladstone *et al.*,

1982). Southern blotting analysis shows that many, although not necessarily all, of the mutagen-induced loss mutants have deletions of some portion of one or more genes comprising the major histocompatibility complex (Orr & DeMars, 1983).

When selection is carried out using monoclonal antibodies, loss mutants that appear similar to those seen with polyspecific antisera are found (Rajan, 1980). However, a new class of mutant is also found that has lost only the specificity selected against and that retains other specificities present on this molecule (Rajan, 1980; Pious *et al.*, 1982). Presumably these cell lines represent single base changes or small deletions whose consequence is a mutant gene that codes for a structurally altered molecule that can be expressed on the cell surface.

Positive immunoselection

The selection protocols discussed so far have been negative selections in which selective pressure is exerted on a cell population expressing a particular molecule. Escape from selection may be either through structural alteration of the molecule such that antibody is not bound or by a mutation whose consequence is a loss of the molecule from the cell surface. Positive selection for surface expression of a molecule by a cell that does not normally express it would be of considerable interest, since there is a high probability that the mutation responsible for such abnormal antigen expression would be in a regulatory gene. This class of mutant has not yet been demonstrated. Perhaps the closest analogy is the isolation of Thy-1⁺ cells from Thy-1⁻ hybrids between Abelson-leukaemia-virus-induced lymphomas and T-cell lymphomas (Hyman *et al.*, 1981). The advantages and limitations of the various techniques available for positive immunoselection have been described by Basch *et al.* (1983).

Cell sorting has been used to isolate mutants that express structurally altered cell-surface molecules. Holtkamp and colleagues first used this approach to isolate mutants for the H-2K^k glycoprotein (Holtkamp *et al.*, 1981, 1983). In this selection protocol, two mutually blocking antibodies were used, one labelled and the other not. Selection was carried out for cells that lost the ability to bind the unlabelled antibody but that still bound the fluorescent reagent. In an alternative approach, Rajan & Halay (1981) sorted for antigen-positive revertants of an H-2K^k antigen-loss mutant isolated by cytotoxic immunoselection. Although the same monoclonal antibody was used both for negative and positive immunoselection, serological evidence suggested that the revertants were probably not identical to wild-type. Structural mutants and revertants isolated in this way have

been particularly useful in studying the nature of the target for T-cell cytotoxic killing (see below).

The T200 glycoprotein is a major cell-surface molecule of haematopoietic cells (Trowbridge, 1978; Sunderland *et al.*, 1979; Omary *et al.*, 1980). A revertant of a T200⁻ mutant was isolated by Hyman *et al.* (1982) by sorting T200⁺ cells from the mutant cell line. The T200 molecule of the revertant cell line showed structural differences compared with wild-type. Evidence indicates that a considerable portion of the wild-type T200 molecule is present intracytoplasmically (Omary & Trowbridge, 1980). The revertant cell line, in contrast, produces a truncated T200 molecule. Structural and peptide mapping studies support the interpretation that the revertant molecule consists of a normal extracytoplasmic domain and a transmembrane region. Most or all of the intracytoplasmic domain of the molecule appears to be lacking from the revertant T200 glycoprotein. The genetic basis for the mutant and revertant phenotype is not yet known; however, the general approach provides a potential means to analyse the function of specific regions of a cell membrane molecule.

Using mutants to study the biosynthesis of cell surface molecules: Thy-1⁻ mutants

One way to study cell-surface-antigen biosynthesis and regulation is to isolate surface-antigen-negative mutant cell lines that fail to express a cell-surface antigen normally present on the wild-type population. Assuming that mutants in different genes affecting the expression of the antigen on the cell surface can be isolated, and assuming that the action of these genes can be analysed, then key steps necessary for expression of the molecule on the cell surface can be defined. These steps represent potential points at which cell-surface-antigen expression may be regulated.

The Thy-1 glycoprotein is a small heavily glycosylated molecule [about 30% (w/w) carbohydrate (Barclay *et al.*, 1976)]. It has an apparent molecular mass on sodium dodecyl sulphate/polyacrylamide gels of 25 000 Da (Barclay *et al.*, 1976; Trowbridge & Mazauskas, 1976), with a true molecular mass of 17 500–18 500 Da (Kuchel *et al.*, 1978). The molecule has been sequenced (Williams & Gagnon, 1982) and both cDNA (Moriuchi *et al.*, 1983; Hedrick *et al.*, 1984) and genomic (Evans *et al.*, 1984) clones have been isolated. There are both structural and sequence homologies with immunoglobulin domains, which has led to the suggestion that Thy-1 may be derived from a primitive cell-recognition molecule and may itself mediate cell-cell interactions (Williams & Gagnon, 1982). The Thy-1 glycoprotein contains three oligosaccharide

structures *N*-linked to asparagine (Campbell *et al.*, 1981; Williams & Gagnon, 1982). It does not appear to be a transmembrane molecule but rather to be coupled to the membrane through a covalently bound lipid moiety (Campbell *et al.*, 1981; Williams & Gagnon, 1982). Although there are considerable species differences in the details of tissue expression (see Campbell *et al.*, 1981), Thy-1 is a major glycoprotein of neuronal cells and, in the mouse, is present in large quantities on thymocytes and peripheral T-cells but is essentially absent from other haematopoietic lineages and from the thymocyte progenitor of the bone marrow (Reif & Allen, 1964; Owen & Raff, 1970; Basch & Berman, 1982).

Using Thy-1 as a prototype differentiation-specific cell-surface molecule, Hyman and colleagues have isolated a number of Thy-1⁻ murine lymphoma mutants. Most of these mutants were isolated by cytotoxic immunoselection on wild-type Thy-1⁺ T-cell lymphomas adapted to tissue culture (Hyman, 1973; Hyman & Trowbridge, 1978; Hyman *et al.*, 1980a). Complementation analysis has been used to ask whether two independently derived Thy-1⁻ cell lines represent the same genetic defect. It is assumed that any two Thy-1⁻ mutant lines with defects in different cistrons will complement in somatic cell hybrids, resulting in cell-surface expression of Thy-1. Hybrids that fail to express Thy-1 on their cell surface are assumed to have genetic defects in the same cistron.

On this basis, eight Thy-1 complementation classes (A-H) have so far been found (Hyman & Trowbridge, 1978; R. Hyman, K. Cunningham & V. Stallings, unpublished results). Complementary hybrids between mutants of Classes A-C, E-F, and H derived from wild-type lines expressing different Thy-1 alleles express both of the Thy-1 alleles characteristic of the respective wild-type lines (i.e. Thy-1.1⁻ × Thy-1.2⁻ → Thy-1.1⁺, Thy-1.2⁺). An analogous result is seen when mutants of these classes are hybridized to Thy-1⁺ wild-type lines expressing the alternative Thy-1 allele. This result suggests that the defects defined by mutants of these classes are in genes whose action is required for expression of the Thy-1 glycoprotein on the cell surface but which do not code for the Thy-1 alloantigenic difference itself.

When mutants of Classes A, B, C, E, and F were biosynthetically labelled and the Thy-1 glycoprotein in detergent-solubilized extracts was isolated by immunoprecipitation and run on sodium dodecyl sulphate/polyacrylamide gels, a structurally altered Thy-1 glycoprotein was seen which migrated more rapidly than the wild-type molecule (Trowbridge & Hyman, 1975; Trowbridge *et al.*, 1978a). The detection of a structurally altered

Thy-1 glycoprotein in these mutants, together with the genetic data, suggested that the genes defined by these mutant classes acted at post-translational steps in the synthesis of the Thy-1 glycoprotein (Trowbridge & Hyman, 1975).

When Class E mutants were biosynthetically labelled with [³H]mannose or [³H]glucosamine and glycopeptides were isolated from Thy-1 glycoprotein, T200 glycoprotein, or the viral gp 69/71 glycoprotein, a decrease in molecular mass of the glycopeptides corresponding to the 'simple' or 'high-mannose' *N*-asparagine-linked oligosaccharides was seen (Trowbridge *et al.*, 1978a). This result suggested that the Class E cell line had a mutation affecting the synthesis of high-mannose oligosaccharides. This mutation affected the structure of many glycoproteins; however, mutant T200 and gp 69/71 glycoproteins were found on the cell surface. Therefore, it appeared that expression of the Thy-1 glycoprotein was particularly sensitive to this change in oligosaccharide structure.

The initial step in the biosynthesis of *N*-asparagine-linked oligosaccharides is the transfer of a preformed oligosaccharide unit from a lipid carrier, dolichol pyrophosphate, to the growing polypeptide chain (Waechter & Lennarz, 1976; Parodi & Leloir, 1979). Therefore, there were two general models for the block in the synthesis of high-mannose oligosaccharides seen in the Class E Thy-1⁻ mutant. The defect could be in the processing of high-mannose oligosaccharides subsequent to the addition of the preformed unit to the nascent polypeptide chain. Alternatively the defect could be in the biosynthesis of the lipid oligosaccharide intermediate.

These alternative models were tested by isolating the lipid oligosaccharide intermediate and showing that it was smaller than the lipid oligosaccharide intermediate isolated from wild-type cells, consistent with the second alternative (Trowbridge & Hyman, 1979). Structural studies (Chapman *et al.*, 1979) showed that the lipid-linked oligosaccharide contained only five mannose residues compared with the nine-mannose-residue structure isolated from Chinese hamster ovary cells (Li *et al.*, 1978) and presumably present in the wild-type lymphoma. This result indicated that the block in the Class E mutant was in a step involved in the addition of the sixth mannose residue to the lipid-linked structure. Alternative explanations for such a block could be lack of the (α1-3)-mannosyltransferase necessary for addition of this residue or lack of the necessary mannosyl donor.

To test these alternatives, Chapman *et al.* (1980) incubated crude microsomal membranes of wild-type and mutant cells with the putative mannosyl donors GDP-mannose or dolichol-*P*-mannose. When GDP-mannose was used, the wild-type cells

formed oligosaccharides containing up to nine mannose residues, whereas mutant cells made oligosaccharides containing no more than five mannose residues. When dolichol-*P*-mannose was used, however, both wild-type and mutant cells formed oligosaccharides containing up to nine mannose residues. This result indicated that the mutant cells have the (α 1-3)-mannosyltransferase required to form the Man₆ (GlcNAc)₂ lipid-linked oligosaccharide, but can express this activity only when exogenous dolichol-*P*-mannose is present. This suggested that the defect in the mutant cell is an inability to synthesize dolichol-*P*-mannose. In support of this interpretation, it was shown that wild-type cells, but not Class E mutants, contain dolichol-*P*-mannose and, furthermore, that wild-type cells, but not mutant cells, synthesize dolichol-*P*-mannose from GDP-mannose and dolichol-*P*. Mixing experiments showed that this inability of mutant cells to synthesize dolichol-*P*-mannose was not due to an inhibitor of GDP-mannose:dolichol-*P*-mannosyltransferase. Therefore, the block in the Class E Thy-1 mutant is most likely to be due to a mutation in the gene coding for GDP-mannose:dolichol-*P* mannosyltransferase with the consequence that this enzyme is not functional in the mutant cell (Chapman *et al.*, 1980).

This analysis of the Class E mutant provides evidence that the expression of glycoproteins on the cell surface is a function of the interaction of specific oligosaccharide structures and particular polypeptide backbones. Thus, cell-surface expression of Thy-1 is normal in a series of pea lectin-resistant and phytohaemagglutinin-resistant lymphoma cell lines (Trowbridge *et al.*, 1978*b*) which have a variety of other defects affecting oligosaccharide biosynthesis (Reitman *et al.*, 1980, 1982; Cummings *et al.*, 1982). Also, the expression of some cell-surface molecules (e.g., Ly-6, H9/25) is sensitive to the Class E defect (Horton & Hyman, 1983), while that of others (e.g., T200, H-2, gp69/71) is not (Trowbridge *et al.*, 1978*a,b*).

This general viewpoint is supported by studies on other molecules. Tunicamycin inhibits the attachment of *N*-acetylglucosamine residues to the dolichol pyrophosphate lipid carrier. Treatment of vesicular stomatitis virus-infected cells with tunicamycin inhibits glycosylation of the viral G protein, leading to a reduction in the yield of infectious virus (Gibson *et al.*, 1978). Although the nonglycosylated form of the G protein can be inserted into the membrane of the endoplasmic reticulum (Rothman *et al.*, 1978) it undergoes aggregation, presumably due to its failure to achieve the normal conformation (Gibson *et al.*, 1979). This aggregation can be partially overcome by growing tunicamycin-treated virus-infected cells at 30°C (Gibson *et al.*, 1979). The yield of

infectious virus in tunicamycin-treated cells is also a function of the strain of virus used, presumably reflecting differences in the sensitivity of particular viral G-protein polypeptide sequence to conformational alterations caused by lack of carbohydrate (Gibson *et al.*, 1979). When vesicular stomatitis virus is grown in wild-type lymphoma cells and in the Class E Thy-1⁻ mutant, temperature effects similar to those observed with tunicamycin are seen (Gibson *et al.*, 1981).

Most of the other Thy-1⁻ complementation classes are far less well understood than the Class E mutant. The exception is the Class D mutant. Hybrids between Class D mutants and wild-type cell lines expressing the alternative allele express the Thy-1 allodeterminant characteristic of the wild-type line but not the Thy-1 allotype characteristic of the mouse strain from which the Class D mutant was derived. This behaviour is consistent with the Class D defect being a mutation in the structural gene coding for the Thy-1 allodeterminant. Southern blot analysis of one Class D mutant and its parent has shown that this mutant has an extensive rearrangement of one copy of the Thy-1 gene with the other, wild-type, allele apparently deleted (Evans *et al.*, 1984). When an intact Thy-1 gene coding for the alternative allele is introduced into this mutant by transfection, the transfected gene is expressed at levels comparable with those of wild-type lymphomas, while the mutant allele is not expressed (Evans *et al.*, 1984).

Using mutants to study the biosynthesis of cell-surface molecules: the role of β_2 -microglobulin in MHC antigen expression

A number of cell lines have been isolated which fail to express Class I major histocompatibility antigens on their cell surface. Human Class I HLA antigens consist of two chains, the polymorphic HLA heavy chain of molecular mass 44000 Da coded for by genes on chromosome 6 and a 12000-Da β_2 -microglobulin chain which is non-covalently associated with the heavy chain and is coded for by a gene on chromosome 15 (Ploegh *et al.*, 1981). The Daudi cell line, a spontaneously occurring human Burkitt lymphoma, was shown to be negative for both cell surface HLA determinants and β_2 -microglobulin. Hybrids of this cell line with RAJI, a β_2 -microglobulin-positive, HLA-positive B cell line, expressed new cell-surface HLA specificities not present on either parent and which were presumably derived from the Daudi cell line (Klein *et al.*, 1977). Fusion of Daudi to mouse cells led to expression of the same HLA specificities; however, human β_2 -microglobulin was not expressed although mouse β_2 -microglobulin was

present (Fellous *et al.*, 1977; Arce-Gomez *et al.*, 1978). This result suggested that Daudi lacked a functional β_2 -microglobulin gene and that expression of β_2 -microglobulin (either human or mouse) was necessary for expression of the Daudi HLA heavy chains on the cell surface. Biosynthetic studies confirmed that Daudi synthesized normal HLA heavy chains but not β_2 -microglobulin (Ploegh *et al.*, 1979). It does not appear that the β_2 -microglobulin gene is missing in Daudi, as normal quantities of β_2 -microglobulin message are made; however, the message cannot be translated (de Preval & Mach, 1983). Thus the data are consistent with Daudi having a mutation in the β_2 -microglobulin gene on one copy of chromosome 15 such that the β_2 -microglobulin mRNA that is synthesized cannot be translated. The other β_2 -microglobulin gene has presumably been deleted, an interpretation that is consistent with the cytogenetic evidence (Zeuthen *et al.*, 1977).

The mutant R1(TL⁻), isolated from a murine thymoma (Hyman & Stallings, 1976), behaves similarly. The parental cell line from which this mutant was isolated expresses murine Class I H-2K^k and H-2D^k antigens as well as the structurally related TL antigens. All these molecules consist of a polymorphic heavy chain coded for by genes on chromosome 17 and a β_2 -microglobulin subunit coded for by a gene on chromosome 2. After selection against TL heavy chain, a mutant was isolated which expressed neither TL nor H-2 determinants. Hybrids between this mutant and an H-2^b, TL⁻ line expressed TL antigen and the H-2 antigens of both parents (Hyman & Stallings, 1977). The mutant did not express detectable β_2 -microglobulin on its cell surface (Hyman & Trowbridge, 1977) nor could β_2 -microglobulin synthesis be detected (Parnes & Seidman, 1982). Southern blot analysis showed that structural changes affecting the β_2 -microglobulin gene had occurred in the mutant (Parnes & Seidman, 1982).

Taken together, the Daudi and R1(TL⁻) cell lines demonstrate that β_2 -microglobulin plays a critical role in the transport of the major histocompatibility antigen heavy chains to the cell surface. This interaction is a function both of the β_2 -microglobulin chain and of the heavy chain itself. This latter point is demonstrated by certain HLA-A2 negative mutants isolated by immunoselection (Kraegel *et al.*, 1982; Pious *et al.*, 1982). These mutants synthesize an abnormal heavy chain which fails to associate with β_2 -microglobulin. The mutant heavy chain is glycosylated but maturation to complex oligosaccharides does not occur. Although this mutant heavy chain can be found inside the cell; no cell-surface expression can be detected. There is a strong suggestion that the primary defect in this mutant is a structural

alteration in the heavy chain such that association with β_2 -microglobulin does not occur.

Exactly why association of heavy chain with β_2 -microglobulin is necessary for cell surface expression of Class I glycoproteins is not clear (Ploegh *et al.*, 1981). There is, however, evidence that the conformation of the heavy chain is considerably different when denatured heavy chain is allowed to refold in the absence of β_2 -microglobulin as compared with when it is allowed to refold in its presence (Lancet *et al.*, 1979). This observation suggests some stabilizing function for β_2 -microglobulin, although additional, regulatory, roles are also possible (Lancet *et al.*, 1979).

Regulation of cell-surface-antigen expression

The studies summarized in the preceding two sections provide examples of post-translational steps that are critical for surface expression of membrane molecules. Whether regulation of surface expression is normally mediated through these post-translational mechanisms is much more of an open question. How tissue-specific expression is regulated at the transcriptional level is even more obscure.

Cell hybridization has been used to study the mechanisms that regulate the expression of cell surface molecules. Several human cell lines, in addition to the Daudi cell line discussed above, have a deficiency in cell-surface expression of β_2 -microglobulin and of HLA antigens. The K562 cell line of the erythroid lineage expresses only low levels of β_2 -microglobulin and of HLA antigens. Hybrids of K562 with the Burkitt lymphoma P3HR-1, a B-cell tumour which expresses both β_2 -microglobulin and HLA antigens, resemble the K562 parent (Klein *et al.*, 1980). This result is in contrast to hybrids of the β_2 -microglobulin-negative mutant Daudi with β_2 -microglobulin-expressing cell lines; in this latter case the hybrid expresses levels of Class I HLA antigen similar to those of the β_2 -microglobulin-positive cell line. Hybrids of K562 with Daudi express more Class I HLA antigens than does K562 itself, although the patterns of expression of individual specificities are complex (Ziegler *et al.*, 1982). These results suggest that the K562 genome may act to regulate expression of the β_2 -microglobulin and/or HLA genes of the partner cell by a mechanism which acts in *trans* position, but the nature of this putative regulation is not known.

The human T cell line CEM also expresses very low levels of Class I HLA antigens relative to B cell lines. Hybrids of this line with B cell lymphomas express levels of CEM Class I HLA antigens comparable with the levels of Class I antigens characteristic of the B-cell parent (Howell *et al.*,

1984). Since the CEM cell line in these studies does not express detectable HLA Class I cytoplasmic mRNA (Howell *et al.*, 1984), it is at least conceivable that regulation is occurring at the level of gene transcription in these hybrids, although regulation at some RNA processing step is not ruled out. These same hybrids also show activation of Class II HLA antigens expressed by neither parent (Howell *et al.*, 1982). The mechanism of this activation is totally unknown; however, it is known that most resting T-lymphocytes do not express Class II antigens whereas stimulated T-lymphocytes often do so. Therefore, the activation of Class II antigens seen in these hybrids might (if one is optimistic) be construed as revealing a normal regulatory pathway. The CEM system seems an attractive one for further study, since the effects seen are large and since nucleic acid probes are available for the molecules being studied.

The possibility that certain regulatory mechanisms for HLA expression may act in *cis* position is suggested from experiments in which microcells containing chromosome 6 derived from an HLA-expressing B-cell $\times$ L-cell hybrid were fused to a mouse embryonal carcinoma line, which itself does not normally express Class I major histocompatibility antigens (Goodfellow *et al.*, 1982). A microcell embryonal carcinoma hybrid synthesized Class I HLA heavy chains, although HLA antigens were not detected on the cell surface, presumably due to a lack of β_2 -microglobulin, which is not synthesized by the embryonal carcinoma cell. Similar studies designed to study developmental regulation of major histocompatibility complex antigens by somatic cell fusions between embryonal carcinoma cells and normal adult cells or cell lines derived from adult animals have led to variable results, depending on the particular combinations examined (Gmür *et al.*, 1980). This shows up the difficulty of interpretation where the interaction of entire genomes is being studied. The expression or nonexpression of a particular molecule at the cell surface may be the end result of the interplay of many genes, making simple interpretations dangerous (see Beckwith & Rossow, 1974, for a discussion of the problems of diploid analysis).

An illustration of the complexity of the somatic hybrid paradigm for studying regulatory control comes from studies on the Thy-1 antigen (Hyman *et al.*, 1981). Hybrids between Thy-1⁺ T-cell lymphomas and Thy-1⁻ pseudodiploid Abelson-leukaemia-virus-induced cell lines, which represent tumours of the early B-cell lineage, show a variable activation of the Thy-1 gene of the pre-B cell parent, whereas the Thy-1 gene of the T-cell parent continues to be expressed. This activation is consistent with a control mechanism which acts in

cis position. When a pseudotetraploid pre-B-cell parent is used as the partner cell, however, neither parental Thy-1 allele is expressed on the surface of the hybrid cell. Thy-1⁺ revertants can be selected from some of these Thy-1⁻ hybrids by cell sorting, but have no obvious change in ploidy. These latter hybrids suggest that a regulatory mechanism exists which acts in *trans* position and whose initial activation is gene-dosage-dependent.

Possibly more amenable to analysis would be somatic cell mutants that identify genes regulating antigen expression and that act at the level of transcription. Class II major histocompatibility antigens consist of two chains of 27 000–29 000 and 33 000–35 000 Da. A series of closely linked genes code for multiple, antigenically distinguishable, chains (Kaufman *et al.*, 1984). A putative HLA-DR regulatory mutant has been described by Gladstone & Pious (1980). This mutant was selected from a heterozygous B-cell lymphoma by selection against one HLA-DR allele. Both the selected and unselected HLA-DR alleles were lost from the surface of the mutant. In addition, other antigens found on chains coded for by closely linked Class II alleles were also not present, suggesting that most or all Class II genes were not expressed on the surface of the mutant. When the mutant was hybridized to a B-cell line carrying a different HLA-DR allele, both mutant HLA-DR alleles were expressed on the surface of the hybrid (i.e. the mutant phenotype was recessive). The exact nature of the defect in the mutant is not known; however, RNA from the mutant is unable to function in a translation assay *in vitro* for HLA-DR light chain and invariant protein (Loosmore *et al.*, 1982). Since the HLA-DR antigens of the mutant are expressed in cell hybrids, this result suggests a defect either in a gene controlling transcription or in some gene involved in RNA processing. Recently, revertants of this mutant have been isolated (Levine & Pious, 1984). These revertants occur at high frequency and tend to be unstable. They also have the rather odd property that, although the revertants were selected by using an antibody that reacts against a nonpolymorphic HLA-DR determinant, only some of the HLA-DR specificities missing from the mutant and present on the wild-type cell line could be demonstrated on the revertant. Levine & Pious (1984) interpret these results to mean that the mutant has a defect in a gene coding for a positive activator of HLA-DR expression. It is postulated that the revertant makes an activator in insufficient amount (or with insufficient affinity) to activate all target genes, thus accounting for the fact that not all wild-type HLA-DR gene products can be detected in the revertants. Whether these speculations are correct awaits more detailed molecular characterization of

these cell lines. Very similar mutants, which lack detectable Class II antigens, have been selected from an independent human B-cell line by Accolla (1983).

A rather suggestive, although still incompletely understood, case of a putative regulatory mutant is the LDHB mouse T-cell lymphoma studied by Holtkamp and colleagues (Holtkamp *et al.*, 1979, 1981, 1983). This heterozygous cell line spontaneously loses K^k expression at high frequency. In many cases the K^k-negative variant cell lines re-express K^k when grown in the presence of γ -interferon (Holtkamp *et al.*, 1979), indicating that, rather than being lost in these K^k-negative sublines, the K^k gene is subject to regulation. In this context it might be noted that Class I mRNA synthesis has been reported to increase when human lymphomas are incubated with γ -interferon (Wallach *et al.*, 1982). A possibly related case is the AKR lymphoma K36, which lacks detectable K^k, although D^k is expressed; in this example K^k is not expressed in somatic cell hybrids between K36 and K^bD^b lymphocytes (Bishop *et al.*, 1982). As both groups point out, H-2K expression is low in normal cortical thymocytes, relative to its expression in medullary thymocytes and thymic emigrants, and it is possible that further analysis of these examples would elucidate normal regulatory mechanisms in the thymus.

Using mutants to study the function of specific cell-surface molecules

Molecules important in immune-cell function

Assuming that functional cell lines are available and that molecules of interest have been identified, then somatic cell mutants provide a way to study how specific cell-surface molecules contribute to cellular function. This approach has been used most by those interested in immune-cell function.

The target for cytotoxic T-cells has been a major issue in immunology. Early on, the R1(TL⁻) mutant (described above), which lacks detectable Class I major histocompatibility antigens, was used to show that presence of serologically detectable H-2 antigens was necessary for specific T-cell mediated lysis and for priming of allogeneic or syngeneic cytotoxic T-cells to minor histocompatibility antigens (Bevan & Hyman, 1977) and to the trinitrophenyl determinant (Dennert & Hyman, 1977). Mutants that have been selected by using antibody against the serologically defined antigens of the Class I antigens of the major histocompatibility complex and that express variant patterns of antigenic specificities have been used as targets in T-cell-mediated cytotoxicity to determine whether the determinants seen by T-

killer cells overlap with the serologically defined determinants detected with monoclonal antibodies. As might be expected given the results with the R1(TL⁻) cell line, mutants that have lost one or more allelic gene products of the major histocompatibility complex are insensitive to lysis by cytotoxic T-cells directed against the missing gene product (Rajan, 1978). The results are quite different when structural mutants that have lost certain antigenic specificities, but still express an altered gene product on their surface, are examined. Vohr *et al.* (1983) studied three K^k mutant cell lines that have lost determinants specified by different monoclonal antibodies but that still express a cell-surface K^k molecule, and found that in nearly all cases the wild-type and mutant cell lines served equally well as target cells. Similar results were obtained by Wraith *et al.* (1983), who showed that a series of K^k mutants could be lysed by an H-2K^k restricted influenza-specific T-cell clone even though lysis of wild-type targets was inhibited by monoclonal antibodies against antigens that were lost in the mutants. These results indicate that the determinants seen by the monoclonal antibodies and the specificities seen by the cytotoxic T-cells are distinct. Potter *et al.* (1983), studying H-2D^d mutants, found that one mutant could not be recognized by a T-cell clone that recognized the wild-type cell; however, another mutant, which appeared serologically very similar to or identical with the first, was lysed. This result also suggests that the determinants defined by antibodies and cytotoxic T-cells are not identical, although they may overlap.

The complementary approach to using mutants made by using monoclonal antibodies to study T-cell function would be to select mutants with cytotoxic T-cells themselves. This approach was first used by Knowles & Swift (1975) who selected against one H-2 haplotype in a hybrid cell line. More recently Maryanski & Boon (1982) have isolated clones of an immunogenic variant line of the P815 mastocytoma that show specific resistance to the selecting cytotoxic cell and are more tumourigenic than is the original variant. Variants of virus-containing AKR leukaemic cell lines that are insusceptible to anti-AKR/Gross virus cytotoxic lymphocytes have been described by Green & Brown (1983).

Wortzel *et al.* (1984) have carried out a detailed analysis of a u.v.-induced fibrosarcoma. This tumour is antigenic and normally regresses when transplanted into intact syngeneic mice. Four antigenic determinants (A–D) recognized by cytolytic T-cells have been defined, although not all tumour sublines express all antigens. Sublines that express only two or three of the rejection antigens were subjected to selection *in vitro* using cytotoxic

T-cell lines that recognize only one determinant. Selection against determinants A or C in A⁺B⁺C⁻D⁻ or B⁺C⁺D⁺ sublines gave variants that grew progressively in animals. Variants of these sublines selected against the B antigen still did not grow in animals. These apparently single-step alterations with profound consequences for the biology of a given tumour provide useful models to study tumour progression (Collins *et al.*, 1980) in a rational way.

One cautionary note is that mutants of tumour cells selected with antibody and complement that show losses of 'normal' cell surface antigens often show changes in the growth properties of the tumours. Buxbaum & Basch (1980) noted that Thy-1⁻ mutants of the tumour RL σ 1 were much less tumourigenic than the Thy-1⁺ wild-type cell line. Thy-1⁺ revertants of this cell line were more tumourigenic than Thy-1⁻ mutants (Basch *et al.*, 1984). Ly 6.2⁻ mutants of the MDAY tumour have been isolated that show alterations from wild-type in growth and metastatic properties *in vivo* (Frost & Kerbel, 1981; Liteplo *et al.*, 1984). These two examples provide evidence that any changes in the cell surface have considerable consequences for the biology of the tumour. Whether the important surface change is in the molecule selected against, however, is not at all clear in these two models, since Horton & Hyman (1983) have shown that certain classes of Thy-1⁻ and Ly 6⁻ mutants have a common post-translational basis which could also affect the surface expression of other, undefined, molecules.

Immune responsiveness to a variety of antigens has been related to a series of loci mapping in the region of the major histocompatibility antigen complex. Glimcher *et al.* (1983) selected what appears to be a structural mutation in the Class II major histocompatibility complex I-A^k molecule in an antigen-presenting cell line and showed that this mutant was unable to present hen egg lysozyme to a hen egg lysozyme-specific I-A^k-restricted T-cell hybridoma. The correlation of the antigenic change in I-A^k with the loss of immune responsiveness suggests that immune responsiveness loci code for the Ia antigens. Functional T-cell hybrids that possess inducible cytotoxic activity are also being studied with the objective of isolating mutants that can define how specific cell-surface molecules contribute to T cell function (Silva *et al.*, 1983).

In this latter context, Lyt-2 negative mutants of cytotoxic T-cell lines have been used to study whether this cell surface molecule is required for T-cell killing. The results both support (Dialynas *et al.*, 1981) and do not support (Giorgi *et al.*, 1982; Kaufmann *et al.*, 1982) this idea, in agreement with the idea that the relative importance of Lyt-2 in T-

cell cytotoxic killing may depend on the particular system examined (Glasebrook *et al.*, 1983).

Molecules that show altered expression during the differentiation of inducible cell lines

The fact that some cell-surface molecules show characteristic changes in their expression during differentiation suggests that these molecules have significance for the cells that express them. One way to study the function of these molecules is to isolate mutants in cell lines that can be induced to differentiate in response to defined compounds. A few examples may serve to illustrate this approach, although none have yet been carried very far.

Friend erythroleukaemias are *in vitro*-adapted tumours of a red cell precursor and can be influenced to differentiate. Induction results in the commitment of the Friend cell to terminal differentiation: haemoglobin synthesis and cessation of proliferation. Upon induction of Friend erythroleukaemia cell lines, an approx. 10-fold decrease in the rate of synthesis of T200 glycoprotein, a molecule whose expression is restricted to haematopoietic cells, occurs by about 3 days (Hyman *et al.*, 1980b). T200⁻ mutants of Friend cells formed haemoglobin to the same extent as wild-type cells, indicating that, while the change in T200 glycoprotein expression is part of the differentiation programme of the Friend cell, presence of T200 glycoprotein is not necessary for the programme to proceed (Hyman *et al.*, 1980b). While this result is the least interesting of the possible outcomes, it does demonstrate the feasibility of using cell-surface mutants to analyse the relationship of particular surface molecules to cellular function.

The mouse pre-B cell line 70Z/3 contains immunoglobulin heavy chain. Incubation with bacterial lipopolysaccharide induces synthesis of light chain and immunoglobulin is found on the cell surface. Mains & Sibley (1983) induced this cell line with lipopolysaccharide and selected against surface immunoglobulin. Two types of mutants were obtained. The first, present at high frequency, could not be induced by lipopolysaccharide. The second, which was much rarer, could be induced but only a proportion of cells synthesized surface immunoglobulin. The exact nature of the defects in these mutant cell lines is not known, but no obvious changes in immunoglobulin gene structure or heavy chain synthesis have been detected. It is possible that the cell lines are not mutants at all but represent stable changes in gene expression leading to a lower probability of differentiation. This situation would be similar to certain variants of Friend erythroleukaemias that do not terminally differentiate in response to inducing compounds (Eisen *et al.*, 1978).

Steroid hormones have a wide variety of cellular effects. After induction of mammary tumour virus-infected rat hepatoma HTC cells by dexamethasone, levels of viral RNA and certain viral proteins are increased as are the levels of various enzymes, among them being tyrosine aminotransferase. One can ask how these various consequences of hormone induction are controlled. To study this point Grove *et al.* (1980) induced the HTC hepatoma line and selected for those cells which showed low levels of the viral gp52 protein by multiple cycles of cell sorting for cells exhibiting low levels of surface fluorescence. Clones derived from this selection showed no or very low levels of induction of either the selected gp52 protein or of the unselected inducible marker, tyrosine aminotransferase. Since levels of glucocorticoid receptor are low or zero in these clones, there is a strong suggestion that the defect in these mutants is at the level of receptor.

Rather different sorts of hepatoma-cell mutants have been isolated by Firestone & Yamamoto (1983). These mutants, selected by multiple rounds of antibody-complement selection against viral cell-surface protein, have glucocorticoid receptors that cannot be distinguished from wild-type by a variety of criteria. One class of mutant shows only a very low level of induction of mammary tumour virus RNA and essentially no induced synthesis of tyrosine aminotransferase, whereas certain other secreted glycoproteins that are normally induced in wild-type cells appear to be induced in this mutant class. Thus, there is at least a suggestion that this mutant is defective in some component involved in the pleiotropic regulation of glucocorticoid-induced responses. A second class of mutant has a defect that is best interpreted as a post-translational defect in processing of the induced cell-surface mammary tumour virus glycoprotein (Firestone *et al.*, 1982; Firestone & Yamamoto, 1983).

Coda: what is the role of the somatic cell geneticist in an era where every gene of any interest is being cloned and subjected to manipulation *in vitro*?

There appear to be at least four areas where somatic cell genetics can make a contribution. In several situations, such as the HLA antigen (Orr & DeMars, 1983) and a series of linked surface antigens on human chromosome 11 (Gusella *et al.*, 1982), molecular probing of deletion variants that have lost particular antigenic specificities permits fine-structure mapping of a particular genetic region. This approach is a straight-forward extension of conventional somatic genetic mapping studies. A second area of focus, which is also relatively easy to rationalize, is the use of somatic

cell mutants to elucidate the post-translational processing of membrane glycoproteins. Several examples have been dealt with in this review and additional discussion may be found in the recent review by Tartakoff (1983). Here, perhaps, it seems most reasonable to concentrate on a few systems where mutants have already been isolated and characterized genetically. The Thy-1 glycoprotein and certain of the viral models seem appropriate for further study.

Although the number of examples is so far limited, somatic cell mutants seem to have something to contribute to the general analysis of cellular function. Further use of mutants to probe immune-cell function, along the lines described above, seems possible. It would also be most interesting if mutants in receptors for specific growth factors could be obtained. Besides allowing study of how the receptor works, certain types of mutants might be very useful as recipients of receptor genes that have been manipulated *in vitro*. Since monoclonal antibodies against many of these receptors are now available, selection of such mutants is feasible, at least in theory. The biggest potential problem is that, since many cell lines that express these receptors require the ligand for growth, mutants that are defective in receptor expression or function may be lethal. It may be necessary to devise selections in which conditional lethal mutants can be isolated. Nevertheless, since structural gene mutants of the transferrin receptor have been reported (Lesley & Schulte, 1984), and since a variety of genetic defects affecting the expression of the low-density lipoprotein have been described (Tolleshaug *et al.*, 1983; Schneider *et al.*, 1983), the situation may not be hopeless.

Finally, and most speculatively, it seems at least possible that mutants might be isolated that identify genes that act in *trans* position and whose products act in the transcriptional regulation of the expression of tissue-specific cell-surface molecules (see above). There is a large literature in somatic cell genetics that says that such genes must exist (see Deisseroth *et al.*, 1980; Ott *et al.*, 1982, for well-studied examples). Working out the details of this regulation for any one cell-surface molecule where molecular probes to the structural gene are also available would more than justify the effort expended.

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