rent exponential trends of population and resource consumption growth. The results showed a rapid depletion of resources and an increase of pollution leading to severe depression of living conditions in the 21st century.

When the model was modified to incorporate in the year 1975 the following changes: (i) zero population growth fertility rates (two children per family), (ii) capital investment set equal to depreciation, (iii) a 75 percent reduction in pollution, and (iv) high capital investment in an agriculture in which there was extensive recycling of materials, a reasonable stability was achieved at high levels of food and industrial output per capita.

However, when these same four changes were instituted in the model in the year 2000 instead of 1975, the equilibrium state was no longer sustainable, and resource depletion caused per capita food and industrial production to decrease rapidly from a high level, starting in about 2050. The drop in population due to impoverishment was projected to begin about 50 years later.

It can thus be concluded that the key to the system of balance is stopping population growth, and the necessary goal must be a solar-powered world. The time to use this key and set out toward this goal must be now—otherwise there may be no such thing as a calamity-free balance between energy and food.

References and Notes

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Surface Modulation in Cell Recognition and Cell Growth

Some new hypotheses on phenotypic alteration and transmembranous control of cell surface receptors.

Gerald M. Edelman

Developmental biologists have long recognized that the evolution of metazoan organisms required the development of special mechanisms to coordinate cell division, cell movement, and cell-cell interactions. Although the precursors of these mechanisms undoubtedly existed in unicellular organisms, the particularly stringent requirements for stable specialized functions within the tissues of multicellular organisms demand much stricter regulation of cellular social behavior and conformity. Nowhere is this more evident than in the patterns of embryonic development of higher organisms (*1*). Even after their removal from mature organisms, cells growing in tissue culture show contact inhibition of movement and density-dependent regulation of growth (*2*). These phenomena reflect the presence of intrinsic phenotypic mechanisms of control that can be genetically altered by transformation of normal cells to tumor cells. The products of such a transformation must affect the various regulatory mechanisms concerned with both cell interaction and cell growth.

All these phenomenological observations point up a problem in the phenotypic behavior of cells that is not simply resolved by such notions as sequential gene programming during development (*3*). This problem is to determine the nature of those cellular structures that regulate division, movement, and cell-cell recognition in such a fashion as to give rise to tissues and organs. It is possible that this complex problem does not have a simple solution despite the evidence suggesting that the cell surface is a major component in the regulatory events (*1*). For example, cell division, movement, and interaction may be under separate regulation by unrelated structures which, like parallel processors in a computer, are coordinated with each other by separate mechanisms at key points in their cycles.

It is my purpose here to propose, on the contrary, that while cell recognition

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in different systems may utilize a variety of mechanisms to achieve specificity, the processes of cell growth, movement, and recognition are all coordinated by an assembly of interacting macromolecules consisting of cell surface receptors and submembranous fibrillar structures. This transmembranous control system depends on the fact that the plasma membrane of the cell surface is a fluid structure (*4*) on and in which surface receptors may move. The lateral mobility of surface receptors provides the basis for several new hypotheses on the molecular mechanisms mediating cell recognition and growth control.

These hypotheses suggest that cell recognition and growth are regulated in large part by epigenetic alterations in the structure of cell surface receptors as well as by changes in their mobility and distribution that lead to modifications of the cytoplasmic structures with which they are associated. Any such change in the structure, pattern, or dynamic state of receptors at the cell surface, I shall call surface modulation (*5*).

The Cell Surface–Membrane Complex and Its Modulation

Specific glycoproteins at the cell surface appear to play a major role in the changes related to surface modulation. Such proteins include many of the cell surface receptors that bind other molecules (for example, hormones, growth factors, antigens) and then mediate a particular function such as mitogenesis, morphogenetic movement, or cell adhesion. Before considering particular examples, it may be useful to consider the situation of a generalized glycoprotein receptor which can diffuse laterally in the plane of the cell surface membrane.

In Fig. 1A are schematically depicted a cell surface glycoprotein and the forces that might act upon it to change its mobility, distribution, or molecular behavior. In accord with several recent studies (*6*), this receptor is shown spanning the membrane (*7*). Because of the complex structure of such a receptor, the simultaneous presence of its parts in heterogeneous phases as it traverses the membrane to the inside of the cell, and its potential interactions with both extracellular and intracellular ligands, the detailed analysis of the forces affecting its motion and distribution is particularly complicated. At present, there are no quantitative estimates of the relative contributions of the various forces acting on such a receptor, but there is some indication that external cross-linkage with other receptors and internal interactions with submembranous structures provide major contributions. While this does not exclude the possibility that certain interactions within the lipid bilayer may play major roles in special cases, the fluid bilayer is viewed here as having mainly a permissive function (*8*).

This point is perhaps most strongly made by considering the result of perturbing (*9*) the cell surface by divalent antibodies to a particular receptor, a process which results in so-called "patch formation" and subsequently "cap formation" (Fig. 1B). Cross-linkage by the specific divalent antibody results in a diffusion-controlled nucleation of the particular receptors binding that antibody. If the cell is metabolizing actively, these cross-linked patches of receptors are then systematically gathered within minutes to one pole of the cell to undergo endocytosis or to be cast off. Although capping depends upon an active process in the cell, it does not require systematic cellular motion (*10*). The position, fate, and nature of the cap do depend, however, upon the morphology, motion, and biochemical state of the cell.

It is important to stress that patch formation is the primary or fundamental molecular process; that is, it reflects the molecular properties of the laterally mobile cell surface receptors embedded in the lipid bilayer. Patching results in surface modulation as does subsequent capping. To date, no physiological function has been found for these modulation events, but their suspected function in stimulating certain processes such as mitogenesis has been effectively excluded (*11*).

Various phenomena that may be related to surface modulation have been described in different animal species, although their connection with patching and capping remains to be made. Antigenic modulation (*12*) in which cellular interaction with antibodies leads to a decrease in a particular surface antigen has been studied in various systems. A similar phenomenon has also been observed in paramecia (*13*). Interaction of antibodies with a specific serological type of surface antigen leads to dis-

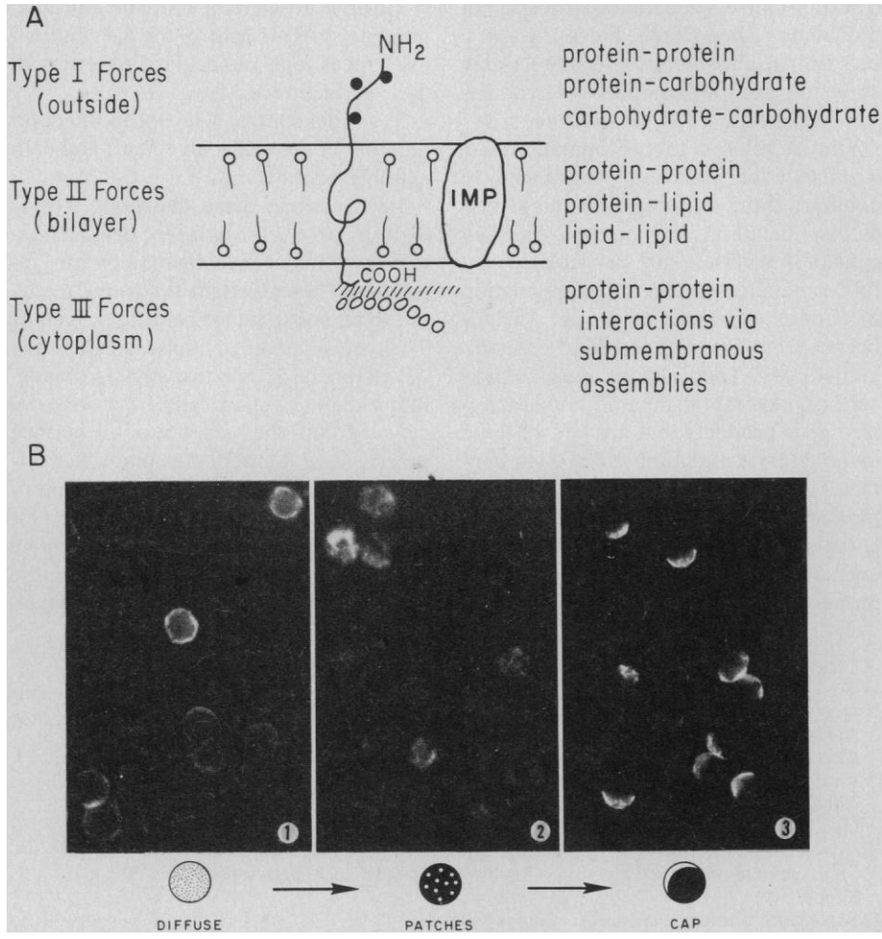

Fig. 1. (A) Schematic illustration of forces that may act on a glycoprotein molecule penetrating the lipid bilayer. Abbreviation: IMP, intramembranous particle. (B) Patching and capping of cell surface receptors. Mouse splenic lymphocytes were treated with fluorescein-labeled rabbit antibody to mouse Ig.

appearance at the surface of that antigen and appearance of antigens of a new type. Viral interactions may lead to alterations in the cell surface including the appearance of new antigens (*14*), a decrease in the amount of surface histocompatibility antigens (*15*), and viral budding at particular sites (*16*). Transformation by oncogenic viruses has been observed to produce alterations in cell surface glycoproteins—for example, disappearance of the LETS (large external transformation sensitive) protein (*17*)—as well as gross changes in surface glycolipids (*18*). Hormonal interactions, such as the binding of insulin, result in alterations of the behavior of insulin receptors on cells as well as in their disappearance (*19*). Fertilization in certain species results in a highly specialized surface alteration of eggs that prevents polyspermy (*20*).

The molecular details and kinetics of many of these modulation events are not, in general, as well understood as those of patching and capping. For this reason, and because cell surface molecules undergo complex cycles of synthesis, shedding, and turnover, it is difficult to propose a general classification of modulation phenomena. Nevertheless, a provisional classification (Table 1) may serve to place the examples and hypotheses discussed here in perspective.

Only in relation to the immune system is knowledge sufficiently advanced to consider these hypotheses in great molecular detail. Lymphocytes, the key cells of this system, are particularly useful for detailed studies of the regulation of various processes at the cell surface because they are readily available as individual cells. They also express at their surface several structurally characterized gene products that are known to be involved in recognition and growth control. Moreover, lymphocytes share many fundamental properties with other differentiated cells, and it is therefore fruitful, even at this early stage, to compare their properties with those of other systems.

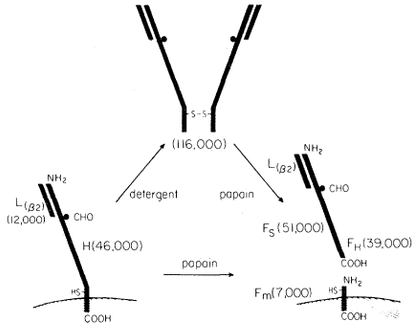

Fig. 2. A model based on chemical studies of the major histocompatibility (H-2) antigen of the mouse (*28*). Numbers refer to molecular weights. The orientation of the molecule was determined by comparing the partial amino acid sequences of H and F_S (*28*). Abbreviations: L, light chain (B_2-microglobulin); H, heavy chain; F_H, heavy chain fragment; F_S, soluble fragment; F_m, membrane associated fragment; CHO, carbohydrate.

With this in mind, I shall consider three questions that are particularly concerned with surface modulation:

1) Does cell recognition by surface receptors during embryological development proceed by mechanisms similar to immune recognition?

2) How do cells of immune and nonimmune systems control the distribution, movement, and molecular properties of their surface receptors?

3) Is this control relevant to the coordination of growth, movement, and cell-cell interaction?

In discussing these questions, I shall emphasize several different cellular systems now under investigation by my colleagues. These systems are (i) the recognition of mouse tumor cells by syngeneic T (thymus-derived) lymphocytes, (ii) the development of cell adhesion and recognition among chick embryonic neural cells, and (iii) the behavior of the surface receptors of lymphocytes and other differentiated cells after the binding of mitogenic lectins. A comparison of the first two systems suggests that the immune recognition of syngeneic cells and the process of cell adhesion during development proceed by quite different forms of local surface modulation that change the structure and specificity of the cell surface. An analysis of the third system points to the existence of global surface modulation and transmembranous control of the distribution and mobility of cell surface receptors, a type of surface modulation that may be concerned with the regulation of growth and other basic cellular functions.

Table 1. Phenomena connected with surface modulation events in various systems.

Noncovalent interactions with local alteration of specific receptors
- Antibody and antigen binding [antigenic modulation (*12*)]
- Alteration of histocompatibility antigens (*23–27*)
- Viral attachment and budding (*16*)
- Attachment of cholera toxin to ganglioside (*61*)
- Insulin binding (*19*) and other hormonal interactions (*2*)

Noncovalent interactions with global alteration of the cell surface
- Cross-linkage of surface glycoproteins by lectins with anchorage modulation (*38, 39*)
- Capping (*9*)

Covalent alteration resulting in surface modulation
- Proteolytic cleavage of surface glycoproteins (*17, 37*)
- Action of glycosyl transferases (*62*)

Complex cellular interactions
- Sperm-egg interaction (*20*)

Local Modulation of H-2 Antigens in Immune Recognition of Cells

It is reasonable to suppose that specific cell-cell interactions in developing tissues are mediated by tissue-specific ligands that are in some way analogous to the antibodies involved in the recognition of the difference between self and nonself. The results of recent analyses of the major histocompatibility antigens are important in considering such ideas if only to cast doubt on them. These antigens also deserve attention here because they are present on cells of various tissues and therefore are among the most convenient surface markers in modulation studies. They are rapidly becoming one of the most extensively characterized of the cell surface glycoproteins, and they therefore provide an important reference for structural and functional comparisons with other surface glycoproteins. Even more to the point, the H-2 antigens are specified by genes at a complex genetic locus (*21*) that is known to be involved in immune recognition and that has been proposed to function in embryonic cell recognition (*22*).

The major histocompatibility antigens, termed HL-A in man and H-2 in the mouse, are a group of surface glycoproteins possessing extensive genetic polymorphism. Many lines of evidence indicate that they are responsible for allograft rejection by cytotoxic T lymphocytes (*21*). It does not appear likely, however, that this is the major function of these surface antigens, for the same types of cytotoxic lymphocytes are also capable of recognizing and destroying virally infected syngeneic cells (*23*) and syngeneic tumor cells (*24*). Indeed, recent studies (*25–27*) suggest that an interaction between H-2 antigens and viral antigens may be necessary for the action of such cytotoxic T cells.

Certain aspects of the structure of H-2 antigens and their association with the cell membrane (*21, 28*) are important in considering their role in surface modulation and cell recognition events. The H-2 antigen of the mouse (Fig. 2) con-

sists of a heavy polypeptide chain (molecular weight 46,000) that carries the distinctive antigenic determinants and a carbohydrate prosthetic group (*29*), and also of a light chain—β_2-microglobulin (*30*)—having a molecular weight of 12,000. The H-2 molecule is probably inserted in the lipid bilayer of the cell membrane because only detergent treatment removes the whole molecule, and soluble fragments of the molecule can be released only by treatment with proteolytic enzymes leaving behind a carboxyl-terminal fragment. Although detergent treatment can result in isolation of a four-chain molecule, in which the heavy chains are linked by at least one S-S bond, it appears that on the cell the two-chain structure is predominant (*28*).

One of the most intriguing features of the H-2 and human HL-A molecules is that the β_2-microglobulin or light chain is homologous in sequence to immunoglobulin (Ig) domains (*30*). Thus, all cells of mammalian species have an Ig-like molecule at their surface. This association of the H-2 heavy chain and β_2-microglobulin is consistent with the possibility that the heavy chain of H-2 antigens may also be homologous to Ig molecules (*31*), although the alternative possibility that this chain has a binding site for Ig-like domains such as β_2-microglobulin cannot at present be excluded. Indeed, this alternative suggests the possibility that the heavy chain may resemble certain proteins of the complement system that also bind to Ig domains. Sequence studies under way (*28*) should resolve this issue. These studies already have indicated that the NH_2-terminus is at the outer portion of the cell surface and thus they imply that the COOH-terminus of the molecule is attached to the membrane.

A clue to the function of histocompatibility antigens was provided by studies (*25*) prompting the hypothesis that these molecules may be modified by viral infection. More recently it has been shown (*25–27*) that the recognition and lysis of tumor cells by H-2 compatible lymphocytes or syngeneic lymphocytes require the participation of the H-2 antigens on the surface of the tumor cells. In functional studies designed to test this hypothesis (*26, 32*), mouse lymphoid tumor cells (P388, histocompatibility type H-2^d) were used to immunize mice of the H-2 compatible strain BALB/c. The interesting finding is that specific antiserum to the H-2^d antigen blocked cytotoxic lysis of P388 cells (Fig. 3A). In contrast, irrelevant antiserums or those against other H-2 specificities did not block this lysis, or blocked it very poorly. Similar results were obtained both with another tumor line, EL4 (H-2^b), and by other workers (*27*) in a different system. An extensive analysis (*32*) has indicated that it is the H-2 antigens on the target cell that are involved in the recognition or killing events. It is particularly significant that H-2 antigens on the target tumor cells can participate in the interactions with the cytotoxic lymphocytes even though these lymphocytes are of the same H-2 type as the target.

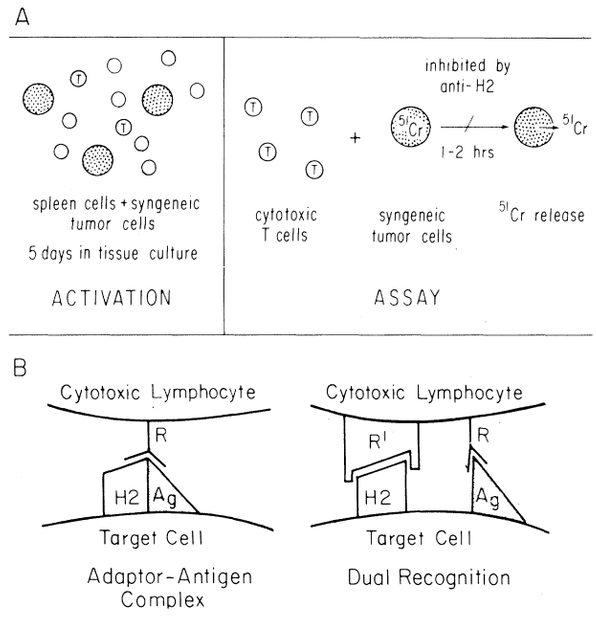

Fig. 3. (A) Assay for demonstrating the participation of H-2 antigens in killing (^{51}Cr release) of syngeneic tumor cells by T lymphocytes (*26, 27*). (B) Alternative models for the immune recognition of tumor cells (*25, 26*). Abbreviations: R, T cell receptor; Ag, tumor viral antigen, R′, second receptor recognizing H-2 antigen.

Two models involving surface modulation (*26*) appear to provide reasonable explanations of these findings (Fig. 3B). Either there is a dual recognition of H-2 and tumor-associated antigens on the cell surface by two separate receptors on the cytotoxic T lymphocytes, or a physical complex is formed between H-2 antigens and tumor-associated antigens on the target and this modulated cell surface complex is recognized by the T lymphocytes. Although the dual recognition hypothesis cannot so far be excluded (*25*), there is some preliminary evidence (*26*) that is in accord with the occurrence of an adaptor-antigen complex containing H-2 molecules (Fig. 3B). This evidence was obtained by using separate antiserums directed against either H-2 antigens or against viral antigens associated with the tumors in order to patch and cap both antigens together on the tumor cell surface. For example, capping and patching of H-2 molecules by appropriately absorbed antiserums that were specific for the H-2 antigen resulted in copatching and cocapping of the viral antigens. Reciprocal experiments in which Rauscher leukemia virus antigens were capped by antiserums directed against the virus resulted in partial redistribution of H-2 antigens into caps.

Although these experiments must be extended and confirmed, particularly to rule out the presence of any unexpected cross reactivity between the various antiserums, they are in accord with the hypothesis (*26*) that H-2 molecules may serve as adaptors that combine with viral antigens in the cell surface to form hybrid antigens containing elements of self (H-2) and nonself (virus). An adaptor-antigen complex may be recognized by a subclass of T lymphocytes having a repertoire of receptors directed against such hybrid antigens. In the adaptor hypothesis, the lateral mobility of receptors provides grounds for specific interactions between glycoproteins on the cell surface and foreign molecules such as those of viruses. This hypothesis provides an example of local noncovalent modification of the cell surface followed by recognition of the modulation by a specific set of antibody receptors on T lymphocytes. This implies an asymmetric interaction between syngeneic cells in which definite, but not absolute, restrictions are imposed by the specificity of the H-2 gene product.

The alternative hypothesis of dual recognition (Fig. 3B) involves surface modulation by viral antigen presentation, but there is no physical interaction of the viral antigen with H-2. In this case, the H-2 antigen may act mainly in the killing step, perhaps by serving as a complement-like surface molecule. The function of β_2-microglobulin may be to modulate the surface by masking and inhibiting this function until cell interaction takes place.

Local Surface Modulation After Proteolytic Cleavage of Receptors

Interactions of neural cells of the chick embryo. One of the most challenging problems of cell recognition is posed by the evidence indicating early establishment of specific cellular connections in embryogenesis (*1*), particularly in the nervous system (*33*). But before problems of this kind can be successfully approached at the molecular level, much work remains to be done on the general nature of cell-cell interaction and adhesion in developing tissues. Various theories (*34*) have been proposed to account for cell interactions of this type, including biophysical theories invoking surface charge differences or differential adhesion and biochemical theories involving specific macromolecular cell ligands that can positively distinguish among differentiated cells from different tissues (*35*). Examples that have been studied range from reassociation in tissue culture of cells from dissociated sponges to an analysis of the interactions of avian and mammalian brain and retinal cells. My discussion is limited to higher organisms, in particular to the retinal cells of developing chick embryos, for recent experiments have shed new light on the means by which these cells associate, and an unexpected form of cell surface modulation appears to be involved.

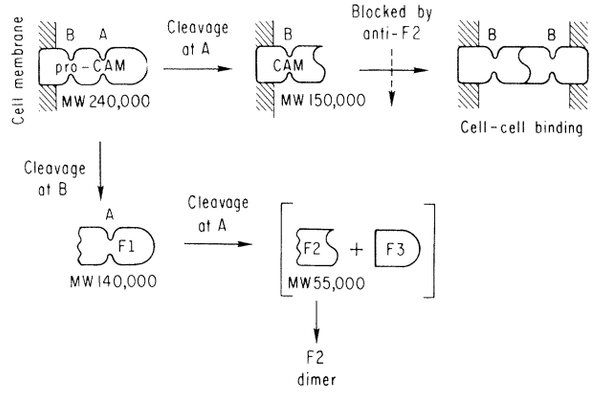

Fig. 4. The CAM-pro-CAM hypothesis for adhesion of embryonic neural cells of the chick. Pro-CAM is the precursor of the cell adhesion molecule, CAM. A and B are sites where limited proteolysis can occur, F1, F2, and F3 are various fragments resulting from proteolysis (*37*).

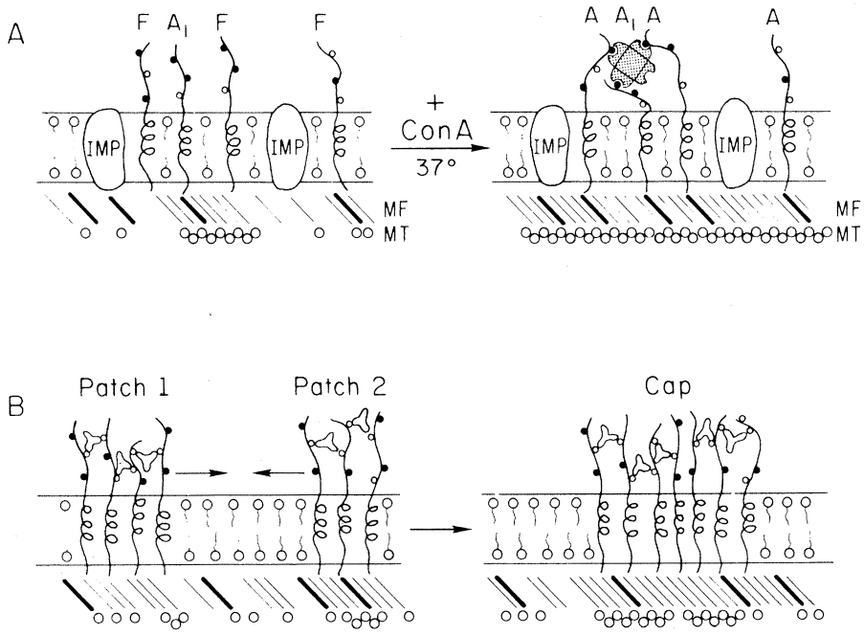

Fig. 5. Anchorage modulation compared with capping. (A) Anchorage modulation. This process is proposed to be mediated by a surface modulating assembly (SMA) consisting of receptors, microfilaments (MF), and microtubules (MT). A_1, preexistent anchored state; A, induced anchored state shown after binding of tetravalent Con A (stippled); F, free state of surface receptors; IMP, intramembranous particles. Heavy bars in MF region represent either cytochalasin-resistant microfilaments or an unidentified anchoring molecule. Cross-linkage of certain glycoproteins leads to propagated anchorage and microtubular assembly. (B) Schematic illustrating induction of caps by microfilaments and associated muscle-related proteins (*9*) independent of the microtubular state. Patches (only two of which are represented) are formed after cross-linking of specific receptors by divalent antibodies shown linking the receptors. Although microtubules have been observed assembling under some caps (*44*), they are not required for the capping process itself. This is in contrast to the essential role of microtubules in anchorage modulation [see (A)].

On the basis of studies that have been carried out on cells from the chick neural retina and other tissues, it has been proposed that each tissue contains a specific soluble aggregating factor that ligates cells of that tissue but not of other tissues (*35*). It has been suggested that these factors interact specifically with cell surface molecules (*36*). In such studies, cells of embryonic chick neural retinas are dissociated after they are treated with trypsin and then grown in tissue culture. Culture supernatants are then assayed for factors that are able, when added to separate cells mixed in suspension, to enhance the formation of large aggregates over relatively long periods of time.

Recent work (*37*) with short-term binding assays to compare the cell surface proteins of retinal cells and brain cells with the proteins isolated from cultures of these cells in different states has revealed a new and unexpected result, however. The interactions of embryonic chick neural retinal and brain cells both appear to depend upon a surface modulation event (Fig. 4) that may involve the proteolytic cleavage of a surface protein to form a cell associating molecule (CAM). The precursor molecule (pro-CAM) of higher molecular weight does not appear to mediate cell-cell interactions. As a result of proteolytic cleavage of pro-CAM by enzymes associated with retinal tissues but not as yet purified, a specific "sticky surface" may be revealed. The simplest hypothesis is that adhesion between two cells results from interaction between these surfaces on the CAM receptors of the different cells. In accord with this proposal, cell-cell interaction may be blocked by specific antibodies to determinants found on CAM derivatives but not on pro-CAM. Moreover, fragments of CAM exist as dimers in solution, apparently interacting via their sticky ends. Two other possibilities have not yet been excluded, however: (i) CAM receptors on one cell bind to an unidentified different receptor on another cell; and (ii) CAM receptors on different cells are bridged by an as yet unidentified molecule.

These experimental results are still very new and must be extended by comparing a number of cellular systems. They do raise the exciting possibility, however, that cell-cell interactions are controlled by surface proteins that arise after serial cleavage of particular cell

surface glycoproteins of high molecular weight. Similar proteins of this kind have been observed on chick brain and chick retinal cells, and it has been shown that these cells can adhere to each other in short-term binding assays (*37*). Under these conditions, the adhesion patterns reflect the age of the tissue, and there does not seem to be a highly specific recognition of cells from these two tissues.

The picture that emerges from these studies is that epigenetic modulation of the cell surface based on protease activity may govern the adhesion of cells in developing tissues. The activation of adhesion by proteolysis of pro-CAM raises the possibility that cell interactions may be controlled by mechanisms similar to those observed in blood clotting and complement fixation. It is possible, therefore, that a cascade of cleavages may lend both refinement and some degree of specificity to such a system. Moreover, enzymes inducing cleavage at sites such as B in Fig. 5 may lead to a reversal of cell adhesion. The CAM-pro-CAM hypothesis is consistent with the idea that the sequential expression of limited protease activity may be one of the key steps in embryonic differentiation.

Although a proteolytic surface modulation mechanism may be necessary for cell-cell interaction in this system, it may not be sufficient. An analysis of the appearance of cells capable of interacting at different embryonic ages reveals that adhesiveness in culture is a function of age and parallels the increase in number of embryonic cells. Cells producing CAM receptors at late stages of development do not interact even though they appear to have the similar numbers of receptors as cells at earlier stages. Other modulation mechanisms may therefore also play a role in cell-cell interaction. An attractive candidate, at least in maintaining specialized surface receptors at a particular portion of the cell surface, is a global form of surface change involving transmembrane control.

Global Modulation of Receptors and Evidence for Transmembrane Control

So far, I have compared two hypotheses invoking different local surface modulation events that result in alteration of the structure of the cell surface and therefore alter cell recognition. In addition, however, an analysis of the molecular interactions of lymphocytes with mitogenic lectins has revealed the presence of a global form of surface modulation that not only affects the anchorage and movement of all receptors (*38*) but also may be involved in the regulation of cell growth and movement.

Table 2. Some tissues, cell types, and receptors showing anchorage modulation (*38*).

Tissues and cells
Splenic lymphocytes
Thymocytes
Thyroid
Kidney
Testis (spermatogonia)
Ovary (cumulus cells)
Polymorphonuclear leukocytes
Cultured fibroblasts
Receptors
H-2 antigens
β_2-Microglobulin
IgG and IgM
Fc receptors
θ-Antigens
Cell surface carbohydrates (including receptors for lectins and antibodies to carbohydrates)

Although the existence of capping implies that the cell possesses structures capable of inducing active movements of aggregates of receptors, it does not reveal much about the anchorage of individual receptors. The key observation relating to reversible anchorage of individual receptors was made when nonsaturating amounts of the plant lectin concanavalin A (Con A), which binds to the carbohydrate portion of various cell surface proteins, were added to lymphocytes (*38*). Under these conditions, subsequent addition and binding of specific antibodies against various receptors failed to induce patches and therefore also did not induce caps. This restriction or anchorage modulation was reversed upon removing the lectin, and it therefore did not result from permanent interference with metabolism or from cell death. Moreover, even local application of Con A to one portion of the cell surface resulted in the restriction of the movement of the receptors on the rest of the cell surface (*39*). This indicates that only a small fraction of the receptors need be bound for all cell surface receptors (Table 2) to be restricted in their movements. Anchorage modulation is therefore a propagated phenomenon (*39*) resulting from amplification of the effects of the initial binding signal and its extension to the whole cell surface. As shown in Table 2, anchorage modulation of a variety of receptors has been studied mainly in lymphocytes, but it also has been observed on a variety of different cells (*38*), with the H-2 antigen used as a marker.

A number of experiments indicate that anchorage modulation is triggered by cross-linkage of certain surface glycoprotein receptors. The effects of cross-linkage can be revealed by modifying the valence of Con A and observing the ability of the derivatives to induce modulation events. Concanavalin A is a tetravalent tetramer consisting of identical subunits capable of binding to glucosides and mannosides, and it therefore probably binds to the nonterminal mannose of the carbohydrate portion of surface glycoproteins. Conversion of Con A to a divalent dimer by succinylation or acetylation (*40*) or to a monovalent dimer by a combination of these techniques with photoaffinity labeling methods (*41*) yields molecules of the same carbohydrate binding specificity that are no longer capable of inducing anchorage modulation. Antibodies against Con A can bind to succinyl Con A that is already bound to the lymphocyte surface and thereby induce modulation. These experiments suggest that anchorage modulation is stimulated by cross-linkage of certain glycoproteins of the cell surface (*39, 40, 42*).

Anchorage modulation appears to be mediated by a collection of submembranous structures rather than by changes at the outside of the bilayer or changes in lipid fluidity. The conclusion (*42*) that this global surface change is mediated by cytoplasmic structures is supported by experiments with drugs that disrupt microtubular structures or interfere with their assembly. If colchicine or various *Vinca* alkaloids are added to lymphocytes in concentrations ranging from 10^{-6} to $10^{-4}M$, anchorage modulation is reversed in many of the cells (*42*), and this effect is itself reversed by removal of the drugs. Inactive derivatives of colchicine such as lumicolchicine, which has no effect on microtubules, do not reverse anchorage modulation (*38*). In experiments with colchicine-resistant cell lines, it has recently been shown (*43*) that colchicine must enter the cytoplasm to affect anchorage modulation. In addition, it has been shown that capping induced by Con A can also induce the redistribution of cytoplasmic microtubules (*44*).

Electron microscopic studies (*38*) suggest that microtubules are not present at the inner lamella of the lipid bilayer, however, and, therefore, even if cell surface glycoproteins penetrate the bilayer into the cytoplasm, some form of linkage between these receptors and microtubules would be required. Possible candidates for this role include certain microfilamentous structures found just under the membrane. It is likely that these actin-like molecules are involved in capping of patched receptors (*45*), for sev-

eral investigators (*38*, *45*) have found that cytochalasin B, a drug that affects certain microfilaments, also inhibits capping. Nevertheless, this drug does not reverse anchorage modulation, in accord with the deduction that some other linkage besides cytochalasin-sensitive microfilaments may be required for the proposed interaction between receptors and microtubules. Aside from cytochalasin-resistant microfilaments, the linkage might consist of various assembly states of tubulin subunits (*38*) or of an additional protein, possibly α-actinin.

On the basis of these observations, various models (*42*, *46*, *47*) have been proposed to account for anchorage modulation. One of the simplest models (Fig. 5) suggests that the appropriate surface modulating assembly (SMA) has a tripartite structure: (i) a subset of glycoprotein receptors that penetrate the membrane and confer specificity on the system; (ii) various actin-like microfilaments and their associated proteins, such as myosin, conferring the properties of coordinated movement necessary for capping; and (iii) dynamically assembling microtubules, both to provide anchorage of the receptors and to allow propagation of signals to and from the cell surface.

In this model, it is assumed that the receptors can exist in two states, anchored and free. Cross-linkage of certain glycoprotein receptors alters the various equilibria between the microfilaments, microtubules, and their subunits and induces a propagated assembly [see (*38*)] of microtubules and fixation of microfilaments. As a result there is a shift to a larger proportion of anchored receptors. Conversely, changes in the state of the cytoplasmic microfilaments and microtubules can alter the mobility and distribution of surface receptors. Disruption of microtubules by drugs (*48*) would still leave the microfilaments and their associated proteins free to induce capping (Fig. 5B).

Studies (*49*) on the disruption of microfilaments and microtubules of mouse 3T3 cells by local anesthetics with concurrent loss of anchorage modulation provide additional confirmation of the basic features of this model. Nevertheless, direct proof of the SMA model is lacking, and alternatives are obviously possible. What is particularly required is a direct chemical demonstration of the postulated molecular interactions among the components of the SMA.

Inasmuch as the states of the fibrillar components of the SMA also affect cell movement (*50*), it is probable that structures responsible for modulating the microscopic mobility of cell receptors are also involved in cell motility and shape change. Certain surface glycoproteins are among the outermost macromolecules of the cell surface–membrane complex and therefore are likely to be in contact with solid substrates and other cells during movement. The carbohydrate portions of such molecules may be the "feet" of the cell, providing weak hydrogen bonding interactions with other cells as well as providing large excluded volumes to prevent other interactions. Anchorage modulation of such receptors would be expected to inhibit motions leading to translocation of the cell, as well as motions leading to alterations in cell shape. A preliminary analysis (*10*) of shape changes in lymphocytes has emphasized the importance of microfilaments and microtubules and indicates that anchorage modulation is, in fact, accompanied by inhibition of cell movements.

Recent experiments provide evidence that the occurrence of anchorage modulation is also associated with blockade of the commitment of lymphocytes to blast transformation and to DNA synthesis. In addition, it has been found that alteration of microtubular states by drugs can influence the passage of this initial mitogenic signal (*11*, *51*). A typical dose-response curve of the mitogenic stimulation of lymphocytes by Con A is shown in Fig. 6 (*11*). Such curves have a stimulatory limb and an inhibitory limb both for DNA synthesis and for cell maturation. The inhibitory limb (which is not due to cell death and which can be reversed under appropriate conditions) occurs in the dose range in which cell surface modulation by Con A occurs. In contrast, divalent succinyl Con A and monovalent succinyl Con A, which do not modulate receptor mobility, show no inhibitory limb (Fig. 6). Synergistic effects of various mitogens of low molecular weight do not alter this basic pattern, which depends on the valence state of the lectin (*52*). A recent experiment (*53*) indicates that release of lymphocytes from modulating doses of Con A still results in commitment to DNA synthesis at normal rates, suggesting that the mitogenic signal is present but inhibited by modulation (*54*).

Additional support for the idea that the SMA, and particularly its microtubular components, may serve as signal regulators for mitogenesis is provided by the

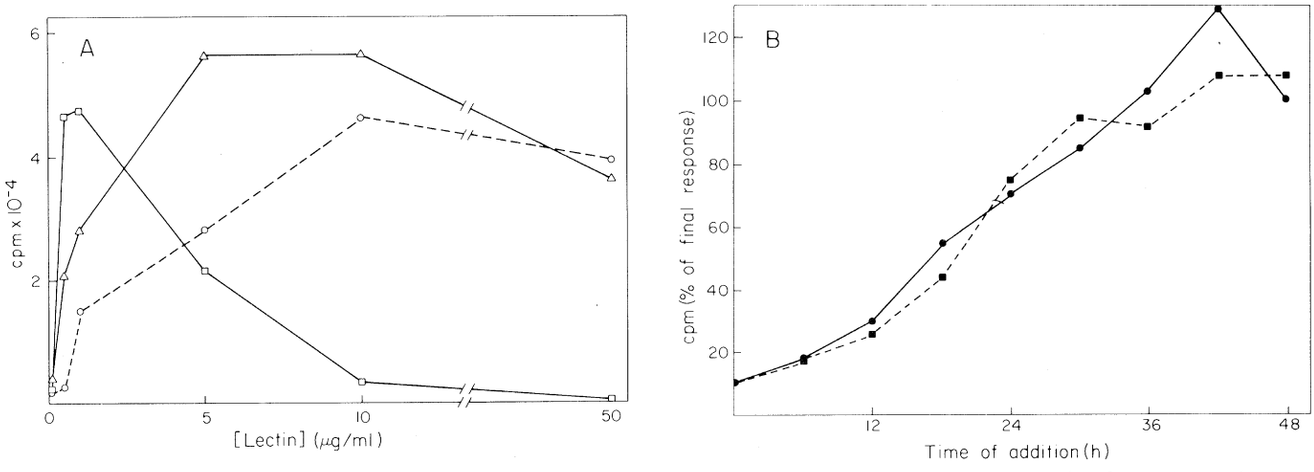

Fig. 6. (A) Mitogenic dose-response curves for concanavalin A and its derivatives of different valence (*41*). □, Native Con A (tetravalent); △, succincyl Con A (divalent); ○, photoaffinity-labeled, monovalent succinyl Con A. (B) Inhibition of the recruitment of cells to undergo mitogenesis. This inhibition occurs after removal of Con A (●) by α-methylmannoside or after addition of $10^{-6}M$ colchicine (■) (*51*). Cells in separate cultures were initially stimulated at an optimal dose of tetravalent Con A [see (A)] and each inhibitory reagent was added at different times after Con A addition. Similar curves are obtained if autoradiography is performed and the number of labeled cells are counted as a function of time. Abbreviation: cpm, counts per minute of [^{3}H]thymidine incorporated into DNA.

finding that the positive limb of stimulation (Fig. 6) can be blocked by low doses of colchicine and related drugs (*51*). It has been found (*55*) that cells are committed by Con A to mature and divide one by one, in an all-or-none fashion. Removal of Con A from the cell surface by competitive binding of a sugar, α-methyl-D-mannoside, stops this recruitment of cells, thereby blocking a mitotic event that would otherwise occur some 20 to 48 hours later. Addition of colchicine without removing the Con A also stops this recruitment (*51*) and with similar kinetics (Fig. 8). Colchicine neither interacts with Con A nor alters its attachment to cell surface glycoproteins.

This kinetic analysis of the commitment of single cells indicates that blockade by colchicine is a very early event and, indeed, it may represent blockade of commitment itself. The blockade appears to be reversible, is induced by other drugs that alter microtubules, and it is not due to interference with the metabolic uptake of precursors of DNA (*51*). The simplest interpretation of these observations is that the microtubular proteins of the SMA are somehow implicated in the regulation of early biochemical signals that induce the cell to mature and divide. In accord with this interpretation, several studies (*56*) suggest that continuously dividing, transformed cell lines have an impairment in their ability to assemble cytoplasmic microtubules. One of the main targets of the products of viral transformation of cells may therefore be components of the SMA, particularly the microtubules.

It is important for the present argument to emphasize that the components of the SMA have just the properties required to serve as signal regulators: (i) SMA components are already phenotypically expressed; (ii) various internal and external states can be communicated via these components, and the responses can be propagated throughout the cell; (iii) alterations in the associated receptor states can affect membrane transport as well as binding of regulatory molecules to various assembly states of tubulin; and (iv) the SMA includes specific receptors, macromolecules involved in cell motion, and macromolecules that may bind various regulators of low molecular weight. The main idea that emerges from these theoretical considerations as well as from the experimental evidence is that components of the SMA may act as negative control elements regulating commitment to cell division, movement, and possibly cell-cell interaction.

Coordination of Signals for Growth Control, Motion, and Cell Interaction

Although anchorage modulation implies transmembrane control over a variety of cellular processes and has global effects on the cell surface, no natural modulating substance capable of inducing anchorage modulation has so far been found. Indeed, the role of soluble substances in growth control has been strongly emphasized (*2*). The possibility still exists, however, that in some cases cell-cell contact (*57*) is responsible for anchorage modulation of all or some receptors in vivo. This touches on the major unsolved problem of morphogenesis (*58*) at the molecular level, the coordination of signals for those fundamental cellular processes that give rise to tissue and organ formation.

A consideration of this problem in terms of modulation events of the kind discussed here immediately raises two difficult questions:

1) How specific must cell-cell adhesion be in order to guarantee orderly signaling?

2) What establishes the priority of the various signals for growth, motion, or cell adhesion?

None of the models presented here directly addresses the question of how specific the cell-cell recognition events are in nonimmune systems, or how specificity is expressed during or after adhesion. This question probably cannot be satisfactorily answered until some of the basic mechanisms of cell adhesion, motion, and growth control are more adequately described. At this stage of our knowledge, it is worth pointing out that, although the cell association model (Fig. 4) does not explicitly include mechanisms related to highly specific recognition, it can easily incorporate them. For example, it is possible that a large number of successive cleavage states of CAM are produced and that these differ in adhesiveness or specificity. Alternatively, noncovalent local alteration similar to that postulated for H-2 molecules could modify the process of adhesion mediated by CAM and lend it some degree of specificity.

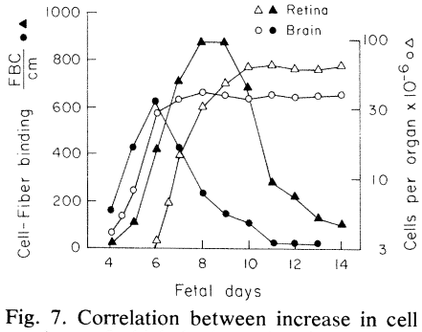

Fig. 7. Correlation between increase in cell numbers of chick brain and retina and the ability of these cells to bind specifically to nylon fibers coated with antibodies reactive with CAM (*37*). The decline in cell-fiber interactions occurs at the time the increase in cell numbers reaches a plateau. Abbreviation: FBC/cm, fiber binding cells per centimeter of fiber.

The problem concerning coordination of signals for interaction, motion, and division is clearly exemplified by the observation (*37*) that age-dependent increases in cell numbers during development are correlated with the adhesiveness of retinal and brain cells to fibers coated with antibodies that bind to CAM (Fig. 7). This correlation is seen despite the fact that CAM receptors are abundantly present on late developing brain and retinal cells. Moreover, a similar correlation is seen for cell-cell interactions among brain and retina cells of different ages (*37*). The decrease in the adhesiveness of late stage cells in tissue culture might reflect the increase with time in the size of a subpopulation of cells in one stage of the cell cycle. At the molecular level, it might also be the result of surface events related to anchorage modulation.

It is tempting to speculate in these terms that a combination of anchorage modulation and covalent alteration of adhesion molecules could account for various adhesive states and mitotic states of higher cellular systems. For example, cells with pro-CAM molecules on their surface would be free to move or divide because no anchorage modulation would be induced in the absence of cell-cell interaction. Induction of proteases leading to CAM formation with subsequent adhesion and possible release of substances inducing anchorage modulation would result in a state in which mitosis as well as movements would be inhibited. Further proteolysis could result in freeing up of anchored cells with the possibility of a further round of pro-CAM synthesis, mitosis (*59*), or movement.

The relation between commitment to mitosis and translatory movement is particularly difficult to establish. One of the conditions accompanying mitosis which would effectively inhibit translatory movement is rearrangement of cytoplasmic microtubules into a distribution that provides no orientation or attachments for microfilaments and their associated muscle-related proteins. During cell division and cytokinesis, the cell undergoes a number of morphological changes involving the cell surface during

which components of the SMA are also likely to be rearranged (*60*). Studies on modulation at different stages of the cell cycle should clarify our understanding of this process and also allow a decision on its relationship to cell-cell interaction.

In view of their needs for specificity and the diverse structural requirements made upon cells in different tissues and animals, it is likely that cell recognition will be found to utilize a variety of different local modulation mechanisms as shown here for the immune and neural systems. On the other hand, the regulatory requirements of cell division, movement, and cell interaction and the exclusive nature of each of these processes would argue for the development during evolution of a general means for their coordination. Transmembrane control seems to have the necessary properties for this regulatory role. Investigation of the molecular mechanism of surface modulation in different systems should enable us to decide whether, in fact, its properties are also sufficient.

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