Intercellular Communication and Cell-Cell Adhesion

S. JONATHAN SINGER

In developmental biology, binary cell-cell interactions often determine the fate of one or both cell partners. The two cells must adhere to one another to allow chemical signals to be transmitted in one or both directions across the regions of cell-cell contact. The molecular mechanisms of cell-cell adhesion and intercellular communication, even if they are mediated by different cell surface components, may be functionally integrated in several different ways. Studies of helper T cells with antigen-presenting B cells in culture have illuminated such binary interactions. The possible application of similar mechanisms to other binary developmental systems is briefly explored.

A WIDESPREAD AND ESSENTIAL PHENOMENON IN DEVELOPMENTAL biology is the determination of the developmental fate of a cell as a result of interactions with its environment. This environmental influence is often provided by specific direct interaction with another cell. A few examples of such two-cell (binary) interactions that are now under intensive study include those occurring during the in vivo development of the *Drosophila* visual system (*1*), of the *Caenorhabditis elegans* vulva (*2*), and of the vertebrate immune system (*3*). In the first two cases, genetic analyses have detected and characterized specific molecules, particularly certain integral membrane proteins, that are critical to the determination of developmental fates of the cells studied. In the case of the immune system, in vitro studies (*4, 5*) have shed light on the molecular basis of certain of these cellular interactions. The molecular mechanisms involved in such binary cell interactions are the focus of this review.

In all such binary developmental interactions, the two cell surfaces are juxtaposed for extended periods of time. It is generally during these periods of cell-to-cell contact (adhesion) that signals are passed between the two cells, in one or both directions. Thus, adhesion and intercellular communication are at the very least correlated phenomena. In the binary interactions that are considered in this review, there is no direct coupling of the two cell cytoplasms (as can occur, for example, through gap junctions); the two cell membranes appear to remain structurally distinct. Adhesion and intercellular communication in such cases must therefore be initiated by the binding of cell surface–associated molecules on one cell to their specific partner molecules on the other (*6*). During adhesion, such transcellular binding must result in chemical signals that are transmitted across intact membranes and that then set off a cascade of reactions within one or both of the contacting cells. Adhesion and signaling phenomena, however, are usually dealt with as two separate mini-worlds of cell biology. Nevertheless, a complex integration of adhesion and signaling events may occur in a wide range of binary cell interactions in development; it is this possibility that motivates this review.

The author is in the Department of Biology, University of California at San Diego, La Jolla, CA 92093.

Mutual Capping and Mutual Co-Capping: The Nature of Adhesion

The long-range mobility of integral proteins within their fluid membranes plays a central role in mechanisms of intercellular adhesion and communication. We therefore begin by considering some relevant aspects of membrane dynamics. The simple binding of a surface molecule on one cell to its partner molecule on the other can often have complex consequences. Under appropriate circumstances it can result in massive redistributions of components in the two contacting membranes, as was first pointed out by Singer (*7*). In the simplest case a cell P has a surface ligand molecule ↑ that can bind to its specific receptor ϒ on cell Q (Fig. 1). If the concentrations of the ligand and receptor molecules in their respective membranes are sufficiently large, and the intrinsic rate constant for the dissociation of the ligand-receptor bond is sufficiently small, then the formation of a small number of ligand-receptor bonds (Fig. 1B) can maintain a localized cell-cell contact long enough for the diffusion of many more ligand and receptor molecules (in their respective membranes) into the contact region where they will form additional bonds clustered within an extended contact area (Fig. 1C). If this clustering (Fig. 1, B to C) does not happen, the cells will come apart again (Fig. 1, B to A). Clustering occurs because the formation of the initial stable cell-cell contact substantially decreases the free energy of formation of later ligand-receptor pair bonds below that of the first pairs. This clustering process is called mutual capping (*8*) by analogy to the antibody-induced clustering of a cell surface molecule that is known as capping (*9*). The hallmark of mutual capping is the increase in concentration of both the cell-surface ligand and its

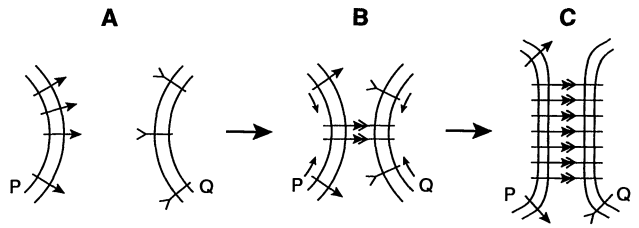

Fig. 1. A schematic view of mutual capping. Under appropriate conditions of ligand and receptor concentrations and affinities (**A**), the formation of a few transcellular ligand-receptor pair bonds (**B**) results in the diffusional recruitment and binding of additional pairs (**C**) to make a stable intercellular adhesion. In the process, the morphology of the cell surfaces in contact (C) may change. See (*8*).

cell-surface receptor molecules precisely within the morphologically defined region of cell-cell contact (*10*).

An important corollary of mutual capping is that changes in the morphology of the two cell surfaces may occur in the cell contact area, subject to constraints on the deformation of the surfaces, particularly by the cytoskeleton underlying each membrane. Such morphological changes may involve a localized flattening together of the two originally curved or convoluted cell surfaces [see figure 5 in (*11*)], or the formation and interdigitation of villous projections of the two cell surfaces [see figure 4 in (*11*)]. The function of such cell surface changes is to maximize the area of close cell-cell contact, which permits a greater number of transcellular ligand-receptor bonds to form across the narrow intercellular gap. The free energy decrease due to the formation of the additional ligand-receptor bonds more than makes up for the free energy required to deform the cell surfaces (*12*).

Mutual capping at its simplest involves a single ligand and its receptor. However, a cell may simultaneously exhibit a variety of independent cell surface ligands and receptors that have their partner molecules on a second, interacting cell. A number of possible processes related to mutual capping might then occur that result in the co-clustering of all or most of these ligand-receptor pairs into the cell-cell contact area; these processes are referred to as mutual co-capping (*8*). A type of mutual co-capping process of particular interest for this review can occur when the cell P′ has two surface ligand molecules ↑ and ● and the cell Q′ has their respective specific receptor molecules ϒ and ϒ, all of which are independent species (Fig. 2). The concentrations of ↑ and ϒ in their membranes, and the rate constant for the dissociation of their bond, are such that these two alone can undergo mutual capping. However, ● and ϒ alone do not satisfy these conditions; their bond might be too weak, or their membrane concentrations too low for ● and ϒ to undergo their own mutual capping. The mutual capping of ↑ and ϒ (Fig. 2, B and C), however, might then induce a simultaneous transcellular binding and co-clustering of ● and ϒ into the same cell contact area formed by the first ligand-receptor pair (Fig. 2D). This mutual co-capping would occur because once a stable cell-cell contact was generated by a sufficient number of transcellular bonds between ↑ and ϒ , the free energy of formation of transcellular bonds between ● and ϒ in the cell contact area would be greatly decreased. The two sets of ligand-receptor pairs would therefore become concentrated and intermixed in the cell contact area, provided there were no significant obstructions to the diffusion of the membrane molecules as the contact area grew. If the stable transcellular binding of ● and ϒ was required to transmit a signal into Q′, the mutual co-capping process would therefore be obligatory for the signal transmission to occur.

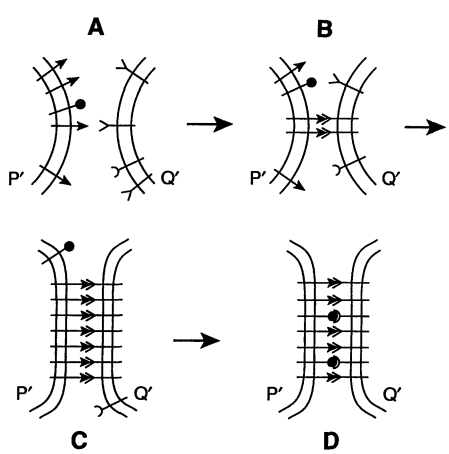

Fig. 2. A schematic view of one type of mutual co-capping of two ligand-receptor pairs (**A**); one pair (↑, ϒ) can alone undergo mutual capping, but the other pair (●, ϒ) cannot. The mutual capping of the former pair (**B** and **C**) allows the latter pair to become bound transcellularly (**D**) in the cell-cell contact site. See (*8*).

Additional factors may operate in individual instances of mutual capping or co-capping (*8*). If stable interactions exist between two integral proteins within a single cell membrane, the clustering of one protein into the cell contact area will necessarily be accompanied by the other. The T cell receptor (TCR) in T cell membranes, for example, is a heterodimer of two polypeptide chains (α and β), which is stably associated with a complex called CD3 that consists of five other chains (*13*). An important variant of such molecular interactions within a single membrane may arise through the transient or induced binding of two or more integral proteins. In such instances, the integral proteins may be ordinarily independent of one another in the cell membrane. However, one of these molecules may be induced to have its conformation so altered that it develops an affinity for the other molecule in the membrane. The TCR in helper T (T_H) cell membranes, for example, on transcellular binding to its cognate ligand, apparently develops an affinity for the CD4 molecule in the same membrane, although TCR and CD4 are normally independent. This affinity is probably caused by a conformational change induced in the TCR upon binding to its ligand. When, therefore, the conformationally altered TCR on T_H cells is induced to cluster, CD4 co-clusters with the TCR into the cell contact region. Such induced co-clustering processes have been called syn-capping (*8, 14*) to reflect their occurrence within a single membrane, and their transient and unidirectional characteristics.

Mutual capping, or closely related phenomena, have now been widely observed experimentally (*15*), and the process has been treated theoretically (*16*). We propose that mutual capping, or more commonly mutual co-capping, processes always occur during the direct molecular interaction of one cell surface with another, unless molecular mobility in the membrane is inhibited. These processes do not just accompany intercellular adhesion; they are primarily what intercellular adhesion is all about. The close contact regions between the two cells arise because of the formation of the mutual caps or co-caps, and the morphologically defined contact regions are coextensive with these caps (*17*). Furthermore, these capping phenomena may have important functional consequences beyond producing adhesion. Mutual co-capping, as already discussed, can provide a mechanism to allow the stable transcellular binding of certain weakly binding ligand-receptor pairs that are thereby allowed to participate in signaling between the two cells. Furthermore, such a clustering of certain receptors in a membrane may itself be critical to signaling processes, since it is well known that in many single-cell systems, soluble growth factors and cytokines activate cells by mechanisms that crucially involve the dimerization or higher aggregation of the receptors to which these soluble ligands bind.

Intercellular Communication

With respect to the physical mechanisms that may be involved in intercellular communication, there are two mechanisms known whereby two contacting cells can communicate with one another in the absence of a gap junction joining their cytoplasms. One is direct, and follows from the binding of a cell surface ligand on one cell to its specific transmembrane receptor on the other, which within minutes may activate a latent enzymatic or regulatory activity in the cytoplasmic domain of the receptor molecule. The second mechanism is indirect, and involves the stimulation of directed secretion from one cell into the narrow intercellular gap where the two cells are in contact. The soluble secreted components then bind to specific receptors on the surface of the other cell, which in turn activates the

receptors. This second mechanism may often require the induction of the synthesis of appropriate secretory components not present in the first cell before its contact with the second, and may therefore require more time and a stable intercellular adhesion to occur.

Direct signaling. Most studies of cell signaling and activation have been on the interactions of soluble small molecule growth factors, agonists, and related ligands with their cell surface receptors, and on the biochemical processes that these interactions generally stimulate, namely, latent enzyme activities and the modification of the intracellular substrates for these enzyme activities. In this article we emphasize the transcellular interactions of cell-surface, rather than soluble, ligands with their membrane receptors, and the physical mechanisms that are coupled to the biochemical processes that follow. Nevertheless, we consider first certain relevant results that have been obtained with soluble ligands, such as growth factors.

Epidermal growth factor (EGF) is a small (53 amino acid residues) soluble protein (*18*) that, upon binding to its receptor, EGF-R [which is a Type I integral membrane protein (*19*)], stimulates the latent tyrosine kinase activity in the cytoplasmic domain of the receptor and sets off a cascade of biochemical events that eventually results in mitogenesis and cell proliferation. Two features of this stimulation are relevant here. (i) Although EGF is monomeric, upon binding to EGF-R it induces the rapid dimerization of the receptor in the fluid membrane of the cell; this dimerization is apparently critical for the expression of the latent tyrosine kinase activity of the receptor (*20*). (ii) Whereas the stimulation of the tyrosine kinase activity is maximal within minutes, the EGF must be bound to its receptor for several hours for mitogenesis to occur. (Removal of surface-bound EGF by specific antibody any time during this period abrogates mitogenesis.)

Epidermal growth factor is synthesized as part of a very large (~1200 residues) membrane-bound Type I integral protein, prepro-EGF (*18*), from which EGF is derived by proteolytic processing. The extracellular domain of prepro-EGF contains within it not only the sequence of EGF, but another eight EGF-like sequences. In certain cells, such as in the kidney, prepro-EGF accumulates without being processed to EGF. It is possible, therefore, that under appropriate physiological circumstances, binary cell interactions might occur in which prepro-EGF molecules on the surface of one cell bind to EGF-R on the second to induce an EGF-like signal into the latter. Indeed, detergent-solubilized but intact prepro-EGF can bind specifically to the EGF-R on cell surfaces and can sustain the growth of an EGF-dependent cell line (*21*). However, whether stimulation can occur in binary cell interactions between transfected cells expressing surface-bound prepro-EGF and cells bearing EGF-R is not known.

A system where such binary cell interactions have been demonstrated to occur involves transforming growth factor–α (*22*). This factor also exists in two forms: a soluble 50–amino acid species (TGF-α), and a larger Type I transmembrane molecule (pro-TGF-α) from which the former is proteolytically derived. TGF-α is homologous to EGF, and can bind to EGF-R and stimulate its latent tyrosine kinase activity. Whether TGF-α also has its own physiologically relevant receptor is not known. Cells transfected with pro-TGF-α express the precursor molecule on their surfaces but form no soluble TGF-α. Such transfected cells specifically adhere to stromal cells that express EGF-R, and the specific transcellular binding of pro-TGF-α to EGF-R induces them to undergo DNA replication and cell proliferation (*23*).

These two sets of results establish that EGF, or TGF-α, while they are covalently part of the internal sequence of their respective membrane-bound precursors, can specifically bind to the EGF-R on the surface of a cell and stimulate the latent kinase activity of the receptor, the same activity that is more commonly induced by the binding to the receptor of the small soluble molecular species split out of these larger precursors. These observations open up a Pandora's box of possibilities. First, there are several other known cases of small soluble growth factors that have membrane-bound precursors (*22*), so it is possible that binary cell interactions involving membrane-bound growth factors are of some general importance physiologically. Second, there is a large and continually expanding family of membrane-bound molecules with EGF-like repeats within their extracellular domains (*24*); a number of these molecules (including *Notch, Delta*, and *crumbs* gene products) are important in development. Although in no instance has any of these EGF-like repeats been shown to interact with, and activate, a receptor molecule, that some may do so is a possibility not to be overlooked.

In the light of these concepts, some interesting suggestions arise with respect to the binary interactions of a cell bearing pro-TGF-α on its surface with a cell bearing EGF-R (*23*). We would predict that if isolated cell couples were examined, the two molecules would be found mutually capped into their cell contact sites. The clustering of the EGF-R that would thereby be induced might be an essential feature of the activation of its latent tyrosine kinase activity, corresponding to the critical role of dimerization of the EGF-R that is induced by its soluble ligand EGF (*20*). Furthermore, in possible cases of physiological interest, where the concentration of pro-TGF-α expressed on a cell surface might be much smaller than that on a transfected cell, the conditions might not be adequate for simple mutual capping of pro-TGF-α and EGF-R to occur. A separate adhesion system might be necessary, so as to induce a mutual co-capping process that is required for the pro-TGF-α to bind to its receptor on the other cell.

Directed secretion. A second mechanism of binary intercellular communication involves the directed secretion of soluble growth factors and related molecules from one cell to its bound congener cell. The existence of this overall mechanism has only recently been recognized. Although in some respects this process resembles the common mechanism of cell stimulation by exogenous soluble growth factors, the special feature is that the stimulation by the soluble growth factors is strictly confined to the congener cell that is bound to the cell performing the secretion. The overall process involves several steps. First, a binary cell adhesion is produced, by some mutual capping or co-capping events appropriate to the two cells. Then, following this adhesion, a polarized signal is transmitted into the secretory cell that induces a reorientation of the perinuclear Golgi apparatus inside that cell to face toward the cell-cell contact (*25*). Vesicles derived from the Golgi apparatus, containing secretory proteins, fuse with the plasma membrane of the cell, thereby releasing the secretory proteins to the cell exterior (*26*). In directed secretion, in part because the Golgi apparatus is first caused to reorient to face the region of cell-cell contact, the secretory vesicles are channeled from the Golgi apparatus to that region of the secretory cell membrane that is in contact with the congener cell. The secretory proteins are therefore released predominantly, perhaps exclusively, into the confined intercellular space. Diffusion of these released secretory proteins out of the intercellular space may occur at only very slow rates, if at all (*27*). If some of these secreted proteins are growth factors, and there are specific receptors for them on the surface of the bound congener cell, the factors may then bind to these receptors in the cell contact region and stimulate their activation. The growth factors would, however, have no effect on other cells in the immediate vicinity. Therefore, a strictly binary intercellular communication is achieved.

How is the Golgi apparatus reoriented inside the bound secretory cell? The mechanism is not known, but probably involves the well-recognized association of the Golgi apparatus with the microtubule organizing center (MTOC) inside interphase cells (*28*). The

two structures are colocalized to one side of the cell nucleus. The MTOC is the structure from which all of the microtubules in the interphase cell grow out. Upon receipt of the appropriate signal emanating from the region of cell-cell contact, it is possible that some of the microtubules, with one end already connected into the MTOC, become attached by their other end to the cell membrane at the cell-cell contact region (*29*). These microtubules may be stabilized by such dual attachment, compared to the other microtubules in the cell that are in rapid polymerization-depolymerization equilibrium (*30*). These stabilized microtubules might then exert a torque or tension on the MTOC. These forces could thereby reorient and perhaps pull the MTOC along with its associated Golgi apparatus towards the cell contact. In the process, the cell nucleus, being surrounded by the microtubular cytoskeleton, could also be repositioned within the cell. Secretory vesicles derived from the Golgi apparatus could then be tracked along the stabilized microtubules attached to the cell membrane at the cell-cell contact, and fuse with only that region of the membrane.

The soluble growth factor proteins required for a particular developmental interaction may not initially be present inside the secretory cell before binary cell adhesion. In that case, the same signal that triggers the reorientation of the MTOC and Golgi, or a different signal parallel to it, would be necessary to induce the synthesis and subsequent secretion of the growth factors after cell-cell contact was made. Depending on the time required for such induction and secretion, the two cells may have to remain in contact for a considerable time.

Secretory components other than growth factors might also be induced and provided by directed secretion only to a congener cell. Specific proteases, for example, might be secreted into the intercellular gap, and act on integral membrane precursors of soluble growth factors (such as prepro-EGF or pro-TGF-α, discussed above), releasing the soluble factors into the intercellular gap to act upon receptors on the surface of the congener cell. If the precursors themselves could not bind transcellularly and activate these receptors, the soluble growth factors released by such localized proteolysis could do so, while restricting the activation to the congener cell.

As a corollary of the process of directed secretion, new membrane mass, derived from the membranes of the secretory vesicles that fuse with the plasma membrane, is perforce inserted into the membrane of the secretory cell where it is bound to its congener cell. This new membrane mass could contain integral protein components that might be involved in additional adhesive or signaling processes that would occur only between the two bound cells. Such additional processes would arise, however, only some time after the initial adhesion was formed.

The Binary Developmental Interaction of T_H Cells and Antigen-Presenting B Cells (B-APC)

Some of the general physical and chemical mechanisms of binary cell adhesion and intercellular communication that are discussed in this review have been encountered in a specific experimental system that involves the in vitro interaction of T_H cells and B-APC of the vertebrate immune system (*4, 5*). This binary interaction is required in vivo for the ultimate differentiation of the B-APC into plasmacytes that secrete soluble antibodies of particular binding specificities.

The B-APC and T_H cells each represent a specific stage in a complex pathway of differentiation of bone marrow–derived and of thymus-derived progenitor cells, respectively. At the particular developmental stage of interest here, B-APC and T_H cells express a different set of cell surface components, which are readily recognized by specific antibodies raised to them. To simplify matters, we refer only to those few of the cell surface molecules indicated in Table 1. These molecules have all been cloned and sequenced (*31*). Each B-APC exhibits a membrane immunoglobulin (mIg) that is a unique (clonotypic) molecule, with a particular binding specificity that is defined by the structure formed by the single variable domains of its heavy and light chains. As the number of potential combinations of such variable domains is enormous, there is a correspondingly large number of closely similar but different potential B-APC. The function of this mIg is to bind to any soluble macromolecule (the antigen) for which its variable domain region happens to exhibit a sufficiently large binding affinity. The antigen-mIg complex is then internalized and processed inside the B-APC. If the antigen is a protein, the processing produces peptide fragments of the antigen, a few of which become tightly associated with molecules of a class II major histocompatibility complex (class II MHC) protein of the cell; this peptide–class II MHC complex is then expressed at the surface of the B-APC, and serves as the antigen-specific ligand that is subsequently recognized in the binary interaction of the B-APC with a particular T_H cell. The mIg, after selectively binding and internalizing an antigen, apparently serves no further role in the T_H:B-APC interaction. Two other related cell surface proteins on the B-APC that are implicated in the binary interaction are ICAM-1 and ICAM-2, which have binding specificity for the LFA-1 molecule on the T_H cell (Table 1).

Table 1. Some surface components on antigen-presenting B cells (B-APC) and helper T (T_H) cells. Additional surface components exist (*4*). LFA-1 is also present on B-APC, and ICAMs on T_H cells, but their involvement in these locations in T_H:B-APC cell interactions has not been established.

B-APC	T_H
Membrane immunoglobulin (mIg)	
Peptide–class II major histocompatibility complex (MHC)	T cell receptor (TCR)
Class II MHC	CD4
ICAM-1	LFA-1
ICAM-2	

The T_H cells also constitute a large set of closely similar cells, each one expressing a unique clonotypic TCR protein on its surface (Table 1). Each TCR has a binding affinity directed to a specific peptide–class II MHC complex ligand expressed on a particular B-APC. That unique binding affinity arises from the structure of the combined variable domains within the α and β chains making up the individual TCR molecule. Relevant to the interaction between T_H and B-APC, all T_H cells also have the CD4 and LFA-1 proteins, referred to as accessory molecules, on their surfaces (Table 1). These are monomorphic molecules, in contrast to the clonotypic mIg and TCR molecules. CD4 is thought to bind to relatively invariant regions on class II MHC molecules (removed from the regions where peptides bind), and to be critically involved in intercellular signaling. LFA-1 is a member of the family of integrin proteins and is an adhesion molecule that binds either to ICAM-1 or ICAM-2 (*4*). Other T_H and B-APC cell surface molecules, including CD2 and LFA-3 (*4*), also appear to play a role in these developmental interactions, but in the interests of avoiding too great a complexity in the following treatment, they will not be considered here.

The developmental interaction of T_H and B-APC is a two-way street. The T_H cells are stimulated by the interaction to proliferate and to secrete soluble interleukins (ILs). These ILs then act upon the B-APC to induce their proliferation and differentiation through several stages into plasmacytes, cells that produce soluble antibodies that possess the same heavy- and light-chain variable domains that were expressed in the mIg of the B-APC from which the plasmacyte

was derived. The primary molecular event responsible for the T_H:B-APC interaction must be the binding of a TCR on an individual T_H cell to the peptide–class II MHC complex ligand on an individual B-APC that happens to have appropriate affinity for that TCR. Because such individual appropriate T_H and B-APC occur only at very low frequencies, early studies of this interaction used mixed populations of T_H and B-APC, and the involvement of specific binary interactions was only inferred. Later, cloned B-APC and T_H cells were derived, as well as B lymphoma cells and T cell hybridomas, that expressed appropriate class II MHC or defined TCR molecules, so that binary T_H:B-APC interactions could be studied directly. The early cell population studies revealed many significant features of the inferred binary interactions, including the critical involvement not only of the TCR and peptide–class II MHC species but also of the CD4, LFA-1, and ICAM accessory surface components shown in Table 1. The accessory molecules were shown to be essential because monoclonal antibodies directed to each of them inhibited or abrogated the IL release or T_H cell proliferation that marked the productive T_H:B-APC interactions.

In order to study directly the binary interaction of T_H and B-APC, individual cell couples formed between cloned T_H and B-APC were then examined by immunofluorescence microscopy to determine the distributions of several cell surface and intracellular components in the couples (*5*). Cloned T_H cells of defined TCR binding specificity, and B lymphoma cells bearing the appropriate class II MHC molecule and pulsed with the appropriate protein antigen, were used to form the cell couples. In the first phase of these studies, several findings were made (*5*) with B lymphoma cells that were pulsed with large amounts of the appropriate protein antigen.

1) *Mutual co-capping*. The individual T_H:B-APC couples showed a substantial enrichment of immunofluorescent labeling for the TCR, CD4, and LFA-1 on the T_H cell membranes precisely where they were in direct contact with the specific B-APC. These results suggested that some kind of mutual co-capping phenomenon had occurred involving these molecules on the T_H cell interacting with appropriate congener molecules on the B-APC, the same T_H molecules that by monoclonal antibody inhibition studies had been previously implicated in the T_H:B-APC interaction. These redistributions in the T_H cell surface were specific; that is, they did not occur if the B cells did not possess the appropriate class II MHC molecule, or were pulsed with the wrong antigen, for the particular T_H used.

2) *MTOC and Golgi reorientation*. The MTOC inside the bound T_H cell, but not that inside the B-APC, had become reoriented to face the region of T_H:B-APC contact, but only in the case of the specific interaction. The Golgi apparatus was presumably also reoriented, because it is invariably linked with the MTOC.

3) *Cytoskeleton involvement*. The cytoskeletal protein talin (*32*) inside the T_H cell, but not that inside the B-APC, was redistributed and found concentrated under the T_H cell membrane where it was in contact with the B-APC. This redistribution occurred only when the interaction was specific. Several other cytoskeletal proteins were not redistributed in the manner of talin.

Further experiments were then carried out to disentangle these several effects. A slightly different T_H:B-APC pair was used that allowed the amount of antigen processed by the B-APC, and hence the amount of peptide–class II MHC ligand expressed on the APC surface, to be varied (*33*). These and other results suggested the following scheme.

1) *Signal-dependent adhesion*. The LFA-1 on T_H cells, and not the specific TCR, is primarily responsible for stable T_H:B-APC adhesion [presumably via transcellular binding of LFA-1 to, and mutual capping with, ICAM-1 or ICAM-2 (*4*)]. However, for this adhesion to form, a specific signal must first be passed into the T_H through a small extent of transcellular binding of some of its TCR with the specific peptide–class II MHC ligand on the B-APC (as in Fig. 1B). This conclusion follows from the finding that in the complete absence of processed antigen on the B-APC, there is no LFA-1–mediated adhesion, despite the fact the LFA-1 molecules on the T_H, and ICAM-1 or ICAM-2 molecules on the B-APC, are present to the same extent with or without the processed antigen. The signal that is received by the bound T_H cell may result in a chemical or conformational change in the LFA-1 molecule that increases its transcellular binding affinity for ICAM-1 or ICAM-2. That signal requires only a small number of transcellular TCR:peptide–class II MHC bonds to form, because the extent of the LFA-1–mediated adhesion is already maximal at low concentrations of peptide antigen expressed on the B-APC.

The same signal induces the association of LFA-1 molecules with talin molecules, previously not linked together, under the membrane of the T_H cell. The cytoskeleton of the T_H is thereby incorporated into the adhesion process.

This low antigen-mediated signal is probably protein kinase C (PKC)–dependent because the association of the normally independent LFA-1 and talin molecules can be induced in isolated T_H cells by treating the cells with phorbol myristoylacetate (PMA), a PKC activator, but only in cells that contain functional PKC (*34*).

2) *Adhesion-dependent signaling*. This low antigen-mediated signal (the first signal), although sufficient to maximally induce LFA-1–mediated adhesion through the mutual capping of LFA-1 with its receptor on the B-APC, is insufficient to reorient the MTOC and Golgi apparatus inside the T_H cell to face the cell-cell contact region, or to activate the bound T_H cell to undergo proliferation. The latter two effects are closely correlated in their signal requirements, but the reorientation is an early event, whereas proliferation occurs much later. The signal to induce them (the second signal) requires larger concentrations of antigen to be processed and presented by the B-APC to the TCR on the bound T_H cell than in the case of the first signal. Only at these larger antigen concentrations, furthermore, do appreciable amounts of the TCR become concentrated in the T_H membrane at the cell-cell contact region, that is, within the same region occupied by the LFA-1 mutual caps. We propose that it is the mutual capping of LFA-1 with its receptor that permits the mutual co-capping of the TCR with its peptide–class II MHC ligand into the cell contact region. In other words, we suggest that in general the TCR cannot directly undergo mutual capping with its specific peptide–class II MHC ligand, either because the transcellular bond between them is too weak, or the concentration of the TCR or of the peptide ligand on the B-APC surface is too small. The mutual co-capping of the TCR and its complex ligand, within the LFA-1–mediated adhesion sites, is critical to the transmission of the second signal into the T_H cell.

How does CD4 come into the picture? CD4 is widely believed to be the molecular species whose transcellular binding to class II MHC triggers the T_H cell to proliferate. However, in the absence of an LFA-1–mediated intercellular adhesion, CD4 does not alone undergo mutual capping with class II MHC, presumably because this transcellular bond is too weak. CD4 may instead become involved through a syn-capping process. When TCR molecules are bound transcellularly by their variable domains to their specific peptide–class II MHC ligands, the TCR may undergo a conformational change such that it acquires an affinity for CD4 within the T_H cell membrane (*35*). The suggestion is, therefore, that when the TCR becomes mutually co-capped with its peptide–class II MHC ligand into the cell-cell contact site, CD4 becomes associated and co-clustered with the TCR, in a syn-capping process. Once sequestered into the cell contact region along with the TCR, the CD4 can then, and only then, bind transcellularly to the class II MHC on the

B-APC; a second signal might thereby be transmitted into the T_H cell. That signal may involve $p56^{lck}$ (*36*), a soluble tyrosine kinase of T_H cells that is non-covalently linked to CD4.

This second signal through CD4 may be responsible for several effects in addition to T_H cell proliferation, or it may be that additional signals are passed between the two bound cells. For example, the clustering of TCR itself, along with its tightly associated five–polypeptide chain complex CD3, into the cell-cell contact region may result in a signal transmitted into the T_H cell. This process would correspond to the activation of isolated T_H cells that results from the clustering of the TCR-CD3 complex that is induced by soluble-specific antibodies (*37*). Such a signal might involve the soluble tyrosine kinase $p59^{fyn}$, which is non-covalently linked to CD3 (*38*).

3) *MTOC and Golgi reorientation and directed secretion.* The B-APC must also be stimulated to proliferate and differentiate as a result of the binary interaction with the T_H cell. The indications are that such stimulation occurs by the directed secretion of ILs from the T_H cell to its bound B-APC.

The second signal required for T_H cell proliferation (or perhaps other signals in parallel) also serves both to reorient the complex of MTOC and Golgi inside the T_H cell to face the cell-cell contact region, and to turn on the synthesis of those ILs appropriate to the subtype of the T_H cell. The reorientation of the MTOC and Golgi in the T_H cell well precedes the onset of IL synthesis, so that when ILs are ready to be secreted, secretion is strictly directed to the bound B-APC. The concentration of ILs inside the T_H cells, as observed by immunofluorescence microscopy, did not peak until 10 hours after the T_H:B-APC couples were formed (*39*). These ILs were present largely inside the reoriented Golgi apparatus facing the cell-cell contact region. These results imply that the specific cell-cell contact, once formed, must be maintained for the many hours required to turn on IL synthesis in the T_H cell and direct it to the bound B-APC, so that only the bound B-APC, and not bystander cells, are stimulated.

This need for a prolonged and stable cell-cell contact may be the reason that the cytoskeleton inside the T_H cell is involved at the cell contact region. The association of talin with LFA-1 may serve to stably link together the LFA-1 molecules that are concentrated in the T_H cell membrane at the cell contact region, and thereby inhibit the disruption of the LFA-1–mediated adhesion.

4) *Reprise.* In the T_H:B-APC binary developmental interaction, several mechanisms contribute to the adhesion and intercellular communication processes. Although carried out primarily by different molecular species, these processes are closely integrated. Signal-dependent adhesion has the useful property that the molecules that are primarily involved in the adhesion between two cells can be widely distributed among many different cell types, but are turned on to promote a particular binary cell adhesion only by a specific signal passed between the appropriate pair of cells. This process not only insures that only the specific pair of cells will adhere, but also that the adhesion is of the same kind and of the same strength, irrespective of the variability of the signaling components that might be involved. This property also provides a mechanism for disrupting the adhesion, by appropriate enzymatic reversal of the effects of the signal. Adhesion-dependent signaling uses signaling components (CD4 and class II MHC) that are common to all T_H clonotypic cells, which are, however, only effective in transmitting a transcellular signal where a strong intercellular adhesion has formed; only a mutual co-capping process dependent upon a separate transcellular adhesion system can bring the signaling molecules together in the cell-cell contact region. Syn-capping processes, in conjunction with mutual co-capping phenomena, can also serve to collect specific signaling components into the contact region. Finally, directed secretion from one cell to its specifically bound congener cell ensures that nonspecific soluble growth factors, perhaps induced in a secretory cell only upon stimulation by signals from its bound specific congener cell, are then released locally at the cell contact to act only on the bound cell.

Possible Applications to Other Cell-Cell Interaction Systems

A major purpose of this review is to suggest that mechanisms of integrating signaling and adhesion processes may have applicability to a wide range of such binary cell developmental interactions. There is no reason to believe, however, that those mechanisms encountered in the T_H:B-APC interaction exhaust the possibilities for integrating adhesion and signaling processes (*8*). Furthermore, certain features of the T_H:B-APC system, such as the existence of clonotypic cell-surface molecules like mIg and TCR, may be unique to the binary interaction. Nevertheless, the material we have discussed here so far may bear on other cases of binary cell development. Only one such binary system is considered as an example. This system involves the interaction between the R8 cell and the pre-R7 cell during the early development of individual facets (ommatidia) of the compound eye of *Drosophila* (*1*). The development of the visual system probably involves many cell-cell interactions, but the interaction between the R8 and pre-R7 system is a particularly clear case of a binary interaction. In the fully developed ommatidium, R8 and R7 are neighboring photoreceptor cells in a cell complex containing six other photoreceptor (R) cells in a precise arrangement. During development of this particular eight-cell system, the R8 cell is the first and R7 the last to become differentiated. The binary interaction with R8 apparently induces the neighboring pre-R7 epithelial cell to become the R7 neuronal cell.

In order to investigate this binary system in vivo (no appropriate in vitro cell culture system has yet been established), molecular genetic technologies have been used. Mutant flies were selected that showed defects in the conversion of the pre-R7 to the R7 cell, without affecting the development of the other cells. One such mutation affects the *sevenless* (*sev*) gene. The Sev protein has an amino acid sequence characteristic of a receptor tyrosine kinase, a Type I integral membrane protein. The Sev protein is expressed in pre-R7 and in several other of the R cells, but not in R8. Its expression in pre-R7 cells is essential to the differentiation of pre-R7 into R7, but its expression in cells other than pre-R7 has no effect on the developmental pattern of the eye. A second gene essential for R7 development is *bride of sevenless* (*boss*). The Boss protein is an integral membrane protein that spans the membrane seven times (*40*), and is a member of the family of such membrane proteins that includes rhodopsin. The Boss protein is expressed only in the R8 cell, and its normal expression is required for the differentiation of pre-R7 to R7. There is evidence that Sev and Boss proteins can bind to one another (*41*), and the possibility exists that a signal is transmitted from the R8 into the pre-R7 cell via the transcellular binding of these two cell-surface proteins.

How is this signal restricted, however, to the pre-R7 cell? The R8 cell makes contact with the other R cells in the cluster that express the Sev protein, in addition to the pre-R7 cell. It is of interest that the Sev protein in the pre-R7 cell appears to be clustered along its border with the R8 cell (*42*), but the Sev protein in the other R cells is dispersed, suggesting that Sev protein in the pre-R7 cells undergoes some mutual capping or co-capping process with surface molecules on R8. Although a number of explanations can be suggested for these findings, one that conforms to mechanisms discussed in this review is that transcellular bonds between Sev and

Boss are critical to the signaling event, but are so weak that they are formed only by virtue of a mutual co-capping process. Some unidentified adhesion molecules on the surface of the R8 and pre-R7 cells would have to be invoked that might be capable of forming a mutual cap and adhesion between only these two cells; the Boss and Sev proteins then undergo mutual co-capping and binding to one another only within this cap. This phenomenon would correspond to an adhesion-dependent signaling process, as described above. The involvement of such postulated adhesion molecules might be difficult to discern by the genetic methods used to screen for mutants that specifically affect only the differentiation of pre-R7 to R7 cells. If such adhesion molecules were ubiquitous in *Drosophila*, its mutant allelic forms might result in many developmental defects earlier in development, and thus go undetected as relevant to the pre-R7 to R7 conversion. The Notch protein (*43*) is a possible candidate for such a widespread adhesion protein. Two members of the integrin family of cell surface adhesion proteins, related to the LFA-1 adhesion molecule of the T_H:B-APC system in vertebrates, are known in *Drosophila* (*44*); they might well be ubiquitous adhesion molecules involved in many binary cell interactions.

In addition to potential adhesion-dependent signaling processes, the possibility of signaling involving directed secretion between R8 and pre-R7 cells, or in some of the other binary cell interactions in the developing eye, should also be considered. Although there is no direct evidence for the involvement of such signaling processes, it is interesting that inside the epithelial cells that are the precursors of the R cells, the nuclei are invariably repositioned from a random to an apical distribution before the cells differentiate into the specific R cells. Neither the molecular basis for, nor the function of, such nuclear movements is known. It was pointed out above, however, that when the reorientations of the MTOC and Golgi occur that are induced prior to directed secretion, they are often accompanied by movements of the cytoskeleton-enveloped nuclei. MTOC and Golgi reorientation processes might therefore be responsible for the nuclear repositionings in the precursor cells (*45*). If there were indeed critical secretory proteins directed from one cell to its binary partner, but these proteins were also used in many other kinds of cell activation processes as well, again genetic screening that was restricted to a particular binary developmental interaction might not detect the involvement of such ubiquitous secretory factors.

These suggestions concerning the R8 and pre-R7 binary system are not meant to be predictive. They are rather meant to be borne in mind when considering binary developmental interactions generally, and in the design of further experiments to investigate these systems.

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