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A possible model for cell–cell recognition via surface macromolecules

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Alternative possibilities for the establishment of the proper cell distribution during embryogenesis are summarized at the beginning, followed by an assessment of the examples known so far where cell–cell recognition is known to be mediated via cell surface components. In the second part the species-specific recognition process which occurs during the sorting-out of dissociated sponge cells is analysed since it may serve as a possible model for cell–cell recognition in higher animals.

Three possible mechanisms for the establishment of proper cell distribution are considered. These include, first, chemotaxis; secondly, guidance of cell or cell sheet movement by extracellular matrix or by surrounding cells and thirdly, random movement followed by recognition at the final point of destination.

Recognition is necessary for both of the two latter processes, i.e. for cell guidance as well as for locking the cells into their final position after random movement. Two basically different recognition mechanisms should be distinguished from each other. On the one hand cells may recognize each other with the help of macromolecules situated in or just outside of the plasmamembrane which fit to each other like enzymes and substrates or antibodies and antigens. On the other hand, cells may exchange information by exchanging cytoplasmatic components via vesicles or gap junctions.

The species-specific aggregation of dissociated sponge cells is considered to be a possible model for cell–cell recognition in higher animals. A proteoglycan-like intercellular macromolecule called aggregation factor seems to mediate recognition of a given species of cells in the reaggregation process of dissociated cells. The data available at the present time suggest that a monovalent surface macromolecule (baseplate) may mediate the recognition process probably by recognizing the carbohydrate side chains of the multivalent proteoglycan aggregation factor.

A cell-free system was devised to mimic this aggregation process. Addition of aggregation factor to baseplate-coated sepharose beads of approximately the size of the original sponge cells has essentially the same characteristics as the cellular system.

Macromolecule-coded surface information for the recognition between cells has not been established during the embryogenesis of higher animals and remains an interesting challenge.

1. Introduction

Little attention has been given to the biochemistry of cell surface components that are not involved in membrane structure or transport systems, but are involved in recognition or adhesion functions between cells.

Some form of cell–cell interaction is a necessary condition for the formation of the intricate cellular architecture of an organ, as well as for the organization and interconnexion of organs.

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Such interactions have to occur, since many cells have a carefully defined position during embryonal development, and since they behave according to their immediate environment, if transplanted elsewhere in the embryo (regulative development). Furthermore, close cellular contacts seem to be necessary for the induction of differentiation, in which information as well as a feedback control of information are probably passed back and forth from cell to cell.

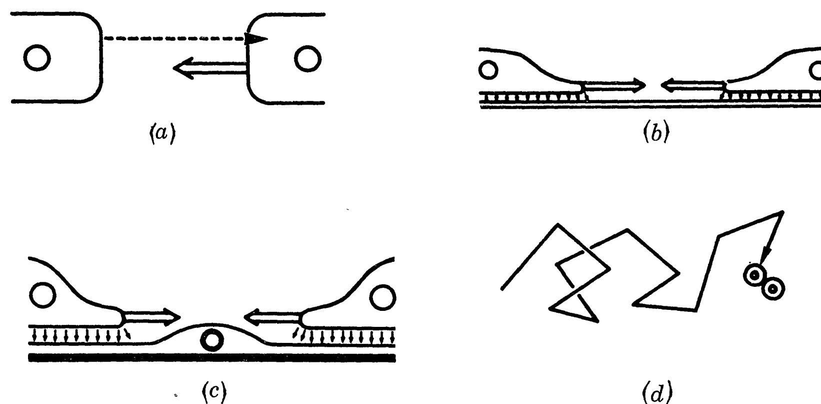

FIGURE 1. Mechanisms by which specific cell–cell contacts can be established (*a*) chemotaxis, (*b*) attraction mediated and guided by an extracellular matrix or (*c*) by another cell, (*d*) random movement and entrapment due to specific cell–cell interaction (from Burger 1974).

If cell–cell recognition is involved in the proper alinement of cells during embryogenesis, then the following theoretical possibilities exist for the establishment of specific cell–cell contacts (Burger 1974): For single cells, the attraction stimulus could be chemical and could be transmitted over large distances, a process generally called chemotaxis (Metz & Monroy 1969) (see figure 1*a*). Alternatively, the attraction could be directed by a specific surface mechanism involving a guideline of extracellular material, e.g. mucopolysaccharides, collagen (Hauschka & Konigsberg 1966), elastin, calcium or silica salts (figure 1*b*). Either single whole cells or networks of cells could serve as guidelines (Sidman 1971), for the directed movement of cells to their final destination (figure 1*c*). Finally, cells can migrate randomly and subsequently form stable attachments only after recognizing the appropriate cell. Such attachments could entrap a cell in a particular tissue or embryo location by virtue of the initially specific surface recognitions (figure 1*d*).

The same theoretical possibilities exist for cell clusters, cell sheets and whole organs. In this case, the interacting cell in figure 1 can be visualized either as a leading cell followed by many other non-interacting cells or it could be substituting for a group of cells, all of which interact with the neighbouring cells. Since many cells in a given organ rudiment seem to be electrically coupled during embryogenesis and since they can exchange small molecules, i.e. possible 'information' through the same coupling, they may interact as a group of cells with surrounding organ rudiments or 'organanlagen', recognizing each other by similar mechanisms as do single cells.

It should be emphasized that recognition between single cells or between coupled groups of cells can occur in principle via two processes. First, via cell surface macromolecules, i.e. a process examined in this article and occurring directly at the cell surface (figure 2, upper portion). Alternatively, the surfaces of opposing cells may temporarily exchange intracellular information at the point of impact either by the exchange of vesicular material or via

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cytoplasmic bridges or, for example, through the much studied gap junctions (figure 2, lower portion). After such a cytoplasmic probing, opposing cells could then decide to establish permanent links or not. The scope of this short review does not permit a discussion of such cytoplasmic recognition processes, but by no means should they be discarded as potentially less important to explain cell–cell recognition *in vivo*.

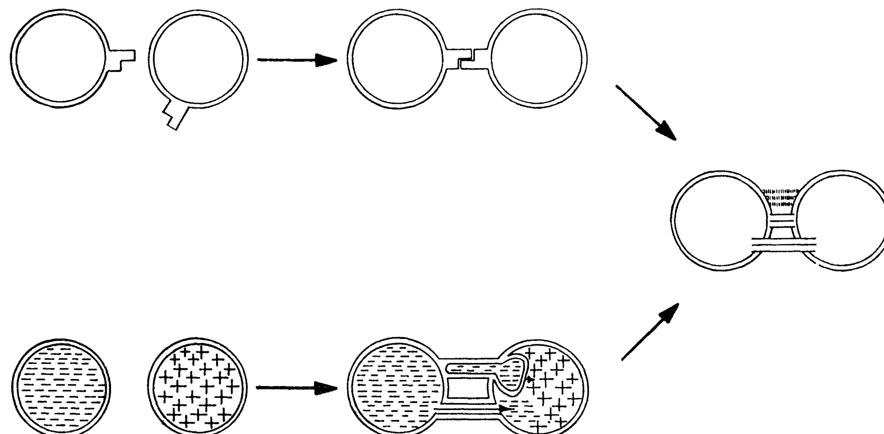

FIGURE 2. Two possible recognition mechanisms. In the upper portion of this figure a mechanism is depicted where recognition occurs directly via surface macromolecules. In the lower portion an alternative mechanism is considered where information from within the cell is passed on to the neighbouring cell, either via vesicular exchange (upper part) or via gap or other junctions (lower part). Such connexions are suggested to have a short life span and can be considered as a mutual probing seen often at the ruffling edge of a moving cell. In both cases such cell interactions will on one hand either lead to secondary stable linkages (intercytoplasmic, intermembranous or extracellular linkages, i.e. lower, middle and upper bridge in the last two adhering cells) or on the other hand to the disengagement of the two partner cells (not shown).

Before leaving the above theoretical argument on cell recognition in morphogenesis and development, it should be pointed out perhaps that behind the question of the mechanism of morphogenesis looms the more formidable question of how the information for the morphogenetic movement is distributed. In other words, what is the original spatial distribution of information in the egg and its relevance to development? Could the original information for organogenesis and morphogenesis be already distributed specifically in the egg cytoplasm and cortex, or is this information read in successive steps from the DNA during the growth and differentiation of the organism?

Cell–cell interactions have been studied at many levels in recent years. In addition to the immune response, which is probably the most popular area of investigation within the field, nerve muscle interactions (Fischbach 1972), fertilization (Metz & Monroy 1969), nerve regeneration (Hunt & Jacobson 1972) and neuronal development (Sidman 1971; Weiss 1970), have been attracting more and more attention, and in a short time these will be subjected to a multi-disciplinary approach. New techniques developed in all of these areas will certainly benefit both the biochemistry and cell biology of cell–cell interactions during development.

The dissociation of organ rudiments with proteolytic enzymes and chelators by the Mosconas (1952) has shown that organ-specific reaggregation results when cell suspensions isolated from several different early chick embryo organs are swirled gently. The relevance of such 'sorting out' experiments is not yet clear. Since many of these sorting specificities are present only during a certain time span in the developing embryo (see §2 and Gottlieb, Merrell & Glaser 1974) and not in the adult, they are thought to be involved in the centripetal forces that are

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perhaps responsible for the formation of the organ in certain instances. They may be required, however, only up to the time when the cells will be fixed together in the proper organ architecture by intercellular bridges, or by a solid capsule surrounding the organ.

In most cases, aggregation *in vitro* unfortunately occurs in two discrete stages: first, unspecific aggregation ascribed to the damage inflicted on the cell surface while the proteolytic dissociation of the embryonal tissue takes place, and then histiotypic sorting out follows as a second stage. This problem of surface damage was later corrected by a modification of the assay (Roth & Weston 1967) although this can doubtlessly be further improved. Furthermore aggregation has since been studied in several invertebrate organisms where dissociation is generally less damaging (Sconto, Pirrone, Mutolo & Giudice 1970; Hynes, Roff & Grass 1972; Gierer *et al.* 1972; Antley & Fox 1970; Fox 1972). Nevertheless trypsin damage remains a serious problem as was pointed out recently when tissue assembly patterns could be shown to vary depending on the time after protease treatment (Wiseman, Steinberg & Phillips 1972). Vertebrate rudiments can also be alined close to one another to form small balls that subsequently engulf each other and lead to onion-like structures that maintain a constant external-internal relation. Steinberg's results with such experiments led him to his 'differential adhesion' hypothesis which offers a purely physical explanation for *in vitro* cellular arrangements. Although his hypothesis is independent of any consideration of the chemistry of the surface constituents involved, he by no means excluded a biochemical receptor mechanism (Steinberg 1970).

Lilien (1969) and Garber & Moscona (1972) have demonstrated that chick retinal and mouse cerebrum cells can release soluble components which specifically enhance the rate and/or degree of reaggregation of dissociated cells from the tissue of origin. Recently, Merell & Glaser (1973) have introduced a novel and interesting procedure for measuring specific reaggregation. Labelled, partially purified plasma membrane preparations were incubated with homologous cultured cells and the amount of label that could not be washed off under mild rinsing conditions was determined. Since even in this system questions can still be raised concerning the degree of damage inflicted on one of the two parts in the reaggregation system (the membrane), and since in all reaggregation assays used to date one still has to ask whether cells that were left (even under optimal conditions) to repair their surface have really regained the same state of differentiation as they had before *in vivo*, we can still expect further improvements in the assays.

2. Slime moulds exemplifying the principle of the optimal developmental stage and surface polarity

The developmental patterns seen in the slime moulds make these organisms ideal for studying the changes in the cell surface during morphogenesis. The life cycle of the cellular slime mould *Dictyostelium discoideum* has two distinct phases; a non-social vegetative state in which separate amoebae feed on bacteria and divide by fission, and a social phase initiated by a period of starvation, in which the free-living amoebae aggregate to form a multicellular structure before fruiting body formation (Gerisch 1968). The initial aggregation after termination of the growth phase is species specific and thus may involve specific cell surface molecules (Raper & Thom 1941). Beug *et al.* (1970) have shown that univalent antibodies directed against discrete cell surface antigens are able to block the formation of intercellular contacts, while having no influence on cell mobility or chemotactic reactivity. Using a different approach, Rosen, Kafka, Simpson & Barondes (1973) have examined the synthesis of a specific carbohydrate-binding

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protein during the life cycle of *Dictyostelium discoideum* and found that the synthesis of this component is correlated with the development of cohesiveness of these cells. This protein, which is assayed by its ability to agglutinate sheep erythrocytes, has a strong affinity for sugars with a galactose configuration. This protein component appears to be present on the surface of cohesive but not vegetative slime mould cells and the developmental appearance of this factor closely parallels the appearance of the antigens reported above by Beug *et al.* (1970). Further characterization of this protein is necessary, as is direct evidence for its involvement in cell adhesion.

A further indication that cell surface carbohydrates may be involved in slime mould development is the preliminary report that there is an alteration in the cell surface during the growth cycle that is detected by agglutination with Con A. Cells harvested from exponential growth phase are agglutinated by lower concentrations of Con A than are cells harvested during stationary growth phase or during differentiation (Weeks 1973). It should be pointed out that the above evidence is only circumstantial and that at present there is no direct evidence for the involvement of surface sugar in this developmental system.

Similar to this alteration in aggregatability during differentiation of slime moulds, certain cell groups may develop optimal affinities for each other during critical phases in embryogenesis, an observation confirmed for organ specific sorting out of cells in higher animals (Gottlieb *et al.* 1974).

If surface affinities between cells should be involved in morphogenesis one would have to invoke asymmetries in the interactions between one cell and different neighbouring cells around it. In other words one cell should not interact with all neighbouring cells to the same extent and in the same manner. Only such polarities within the surface of a given cell could eventually give rise to the intricate forms of organs and the architecture of tissues within an organ, all of which usually deviate from a perfect sphere.

Beug, Katz & Garisch (1973) and Beug, Katz, Stein & Gerisch (1974) found such a polarity involved in the aggregation of *Dictyostelium discoideum* cells. During the aggregation process cells are elongated and show a preference for end-to-end contacts although they adhere also side-by-side to each other. Thus they form stream-like assemblies. Univalent antibodies against surface antigens occurring only in aggregation competent but not the vegetative amoeboid state, abolish the preference for end-to-end assembly. Cells aggregate therefore loosely and side-by-side only. Univalent antibodies against the antigens that occur also in the vegetative state, abolish the side-by-side contacts, still permitting end-to-end contact and assembling the cells into rosette contacts and long, single row cell chains.

A wealth of forms can be generated by stipulating gradients of interaction sites over the cell surface or by considering several qualitatively different interaction sites at various locations on the cell surface.

3. Cell–cell recognition in gametes as a general model

Many simple eukaryotes have flagellated gametes which mate only if they do not belong to the same mating type strain. Not only the male type gamete but also the female type gamete carries flagella and the initial interaction is believed to be due to some sort of agglutination between the two types of flagella.

Thus for *Chlamydomonas*, Wiese (1965) has shown that flagella tips or isolated isoagglutinins

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from one mating type can agglutinate cells from the opposite mating type. These isoagglutinins appear to be high molecular mass glycoproteins. It is interesting that only the female gamete appears to be sensitive to low doses of trypsin and only the male gamete can be inactivated after binding to low concentrations of the agglutinin Con A (Wiese & Shoemaker 1970). Thus it appears that the mating adhesion is brought about by an interaction of a carbohydrate component on the surface of the male gamete and a protein component of the female gamete. Such conclusions are tentative and require considerably more biochemical evidence. Thus, the inhibition of the mating agglutination by Con A may simply be due to steric effects by the bulky Con A.

A similar system is now available in yeast mating interaction. Crandall & Brock (1968) have partially isolated some glycoproteins from the surfaces of opposite yeast mating types which agglutinated or neutralized the opposite mating type. These surface components as well as those in the *Chlamydomonas* system mentioned above, require better chemical characterization to clearly establish their direct involvement in the mating interaction.

4. Sponge reaggregation as a biochemical model

Investigations of the reaggregation of sponge cells have several advantages over similar studies on vertebrate cells. First, the sponge tissues can be dissociated simply with calcium-magnesium free seawater and do not require proteolytic enzymes, that unfortunately have to be used for all of the work with vertebrate tissue. This then allows experiments on essentially intact cells. Secondly, the aggregation factor can be isolated more reliably and in sufficient amounts for large scale biochemical analyses.

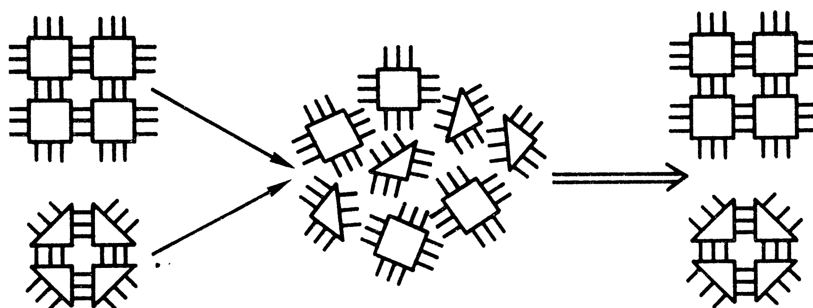

Figure 3. Specific sorting out of sponge cells. When two unrelated species of sponge tissue are mechanically dissociated into single cell suspensions and mixed, they will in due time sort out into two separate sponge cell clumps of the original species. The rectangular species can be, for example, *Microciona prolifera* and the triangular species *Haliclona occulata* while the spikes represent the hypothetical surface material necessary for specific reaggregation (from Burger 1974).

Guided by suggestions made by Andrews (1897*a*, *b*) that a viscous surface substance could enhance the establishment of contacts between 'like' cells, Wilson (1907) chemically dissociated two different sponge species collected at Woods Hole, mixed them and found that aggregates that subsequently formed only contained cells from a single species. This clever experiment, using colour as a marker for the two species, instead of isotopes as one would use today, suggests that two different sponge species must have a way of recognizing their own from different cells – presumably via cell surface components (figure 3).

These findings were later confirmed by Galtsoff (1925*a*, *b*), as well as extended to include the

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observation that reaggregation is dependent on the presence of calcium. DeLaubenfels found, however, also some interspecies mixing where the cells were dissociated in the same bolting cloth (1928). Humphreys (1963) and Moscona (1963) made good use of this information by developing a gentle dissociation procedure consisting of a calcium-magnesium free seawater treatment which releases a large molecular mass factor from the sponge tissue. This material called aggregation factor (AF) from now on, promoted species-specific aggregation of the dissociated cells (figure 4).

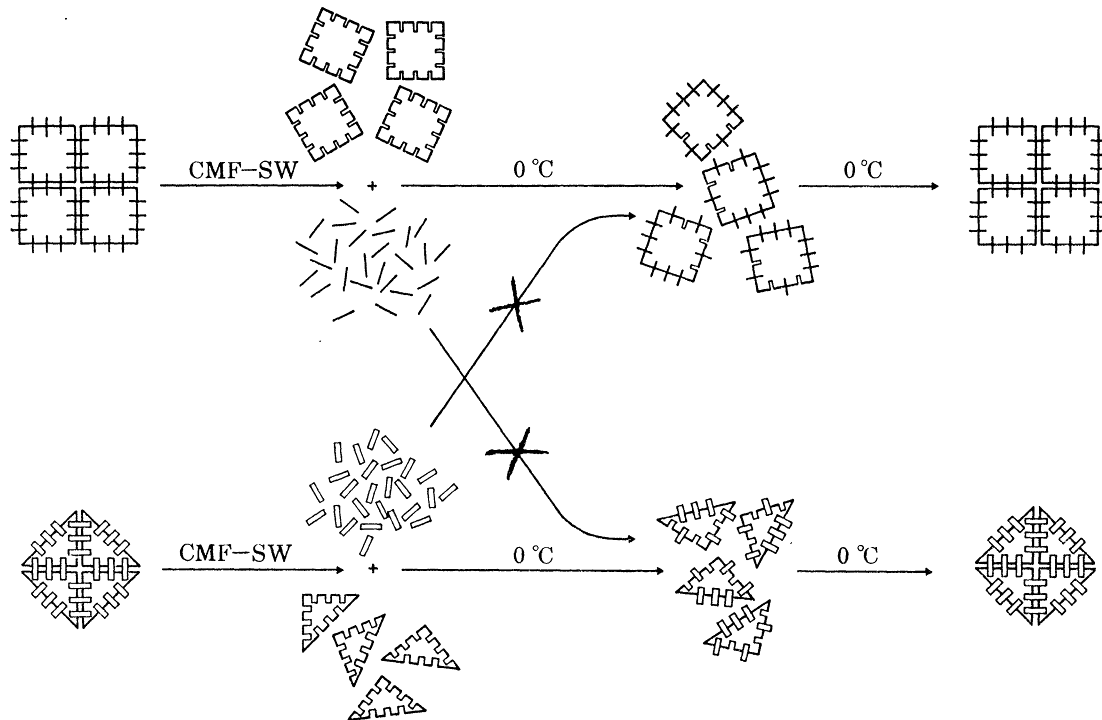

FIGURE 4. Promotion of species-specific aggregation by isolated aggregation factors. Calcium-magnesium free seawater (CMF-SW) causes the release of a species-specific factor which promotes reaggregation of its own kind only if the species investigated are not too closely related.

However, the specificity of the aggregation factor does not seem to be absolute, as was first pointed out by MacLennan & Dodd (1967) by showing that a close taxonomic relationship of 3 sponges decreases their specificity barriers, an observation which we have quantitated and confirmed (Turner & Burger 1973). It should be pointed out, however, here that comparisons of factor specificities should not be done in assays which essentially measure end point or equilibrium conditions of aggregation. In other words, aggregation kinetics, i.e. initial velocities of aggregation should be compared using low amounts of aggregation factor. Under such conditions species-specific differences with different aggregation factors and the same factor free cells should be seen much earlier. This criticism holds for most of the literature including our own work (Turner & Burger 1973).

Taxonomic considerations were also brought forward in recent autoradiographic analyses of reaggregated, mechanically dissociated cells (McClay 1971). Since sponge taxonomy is far from being well established, and furthermore since the technique for determining specificity may have to be re-evaluated (Curtis & Van de Vyver 1971), the details of such questions remain to be resolved.

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The chemical nature of the species-specific aggregation factor was first analysed by indirect procedures as for example with the help of degradative enzymes (Gasic & Galanti 1966; Moscona 1968), as well as by other procedures (Margoliash *et al.* 1965), and it was found to be a proteoglycan. A very careful and detailed analysis of a specific *Microciona* (*M. parthena*) aggregation factor comes to the conclusion that it is a large heterodisperse 70 S complex with a molecular mass averaging about 2×10^7 (Cauldwell, Henkart & Humphreys 1973). After removal of Ca^{2+} and irreversible dissociation, 9–10 S subunits with a molecular mass of about 2×10^5 could be isolated that seem to have about the composition of the native aggregation factor. 47 % consisted of protein and 49 % of carbohydrate, a fifth of which was uronic acid (Henkart, Humphreys & Humphreys 1973). *Microciona prolifera*, the factor used in our study, similarly had a ratio of protein to carbohydrates of 1:1 and equally contained some uronic acid although somewhat less (10 % of the total carbohydrate).

5. Indication for the involvement of carbohydrate in a species-specific sponge aggregation process

If carbohydrates are involved in the specific reaggregation process in a manner analogous to an antigen in an antibody–antigen reaction, then one could expect that certain carbohydrates and their derivatives should be able to inhibit reaggregation in the manner classical haptens do.

A few years ago we reported that the reaggregation of *Microciona prolifera* cells induced by the homologous aggregation factor could be inhibited with glucuronic acid, as well as by cellobiuronic acid, a disaccharide derivative of glucuronic acid (Burger, Lemon & Radius 1971). This inhibition turned out to be quite specific, in so far as none of the other available sponges could be inhibited by this particular sugar, and since no other carbohydrate displayed any inhibitory activity towards this particular sponge. Since Ca^{2+} was important for the stability of the aggregation factor and since the aggregation did not occur in the absence of Ca^{2+} the criticism could be raised that glucuronic acid might simply sequester Ca^{2+} due to its ability to chelate Ca^{2+}. Such an artefact can be ruled out in view of the fact that galacturonic acid does not inhibit reaggregation at all, although it binds Ca^{2+} at least as well, if not better than glucuronic acid. Furthermore, the addition of excess Ca^{2+} did not overcome the inhibition by glucuronic acid (Turner, Weinbaum, Kuhns & Burger 1974).

Based on Gasic & Galanti's (1966) observations that disulphide groups in the aggregation factor are crucial and that the factor loses its activity when incubated with proteases, it was generally assumed that factor activity resides in the proteinaceous moiety, although the presence of the carbohydrate moiety of the aggregation factor has never been subject to doubt. In view of the carbohydrate inhibition data cited above, however, we interpreted these observations differently. Agents that destroy the protein portion of the aggregation factor might, in many cases, dissociate the protein core of the carbohydrate carrying subunits, thereby leading to the formation of monovalent or non-functional oligovalent pieces of aggregation factor. Small amounts of such pieces would not only have lost their aggregation capability for cells but they could impede aggregation by binding as 'monovalent' pieces to their respective binding sites on the cells, thereby preventing the remaining active aggregation factor molecules from binding to and aggregating the cells.

This then led us to the concept (Weinbaum & Burger 1971, 1973) that the cell surface might carry a receptor molecule that probably is a protein, capable of recognizing carbohydrates on

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the aggregation factor. For *Microciona prolifera* this factor then might have antigenic, rather than antibody-like function, as was generally assumed previously.

Evidence that *Microciona prolifera* aggregation factor carries the antigenic carbohydrate essential for the recognition process can be summarized by the following (Burger *et al.* 1971; Turner & Burger 1973):

1. A *Helix pomatia* mixture of glycosidases containing, among others, glucuronidase is capable of destroying aggregation factor activity after preincubation at 37 °C, but not at 0 °C. This inactivation can be prevented with high concentrations of glucuronic, but not galacturonic acid. Such product inhibition of glycosidases is well known, and is spread quite widely within this class of enzymes. Pure β-glucuronidase preparations do not inactivate aggregation factor, an observation which is interpreted to mean, among other possibilities, that glucuronic acid can be protected by some other sugars at the end of the chain and that these groups would first have to be removed before glucuronic acid can be removed.
2. Preincubation of glucuronic acid with the dissociated cells is much more efficient in preventing aggregation than is preincubation of the aggregation factor with glucuronic acid.
3. Preliminary studies by Kuhns (Kuhns & Burger 1971; Turner *et al.* 1974; Kuhns, Weinbaum, Turner & Burger 1974) with aggregation factors isolated from several different sponge species indicates that the aggregation factor from *Microciona prolifera* can be inhibited by several plant lectins specific for more than one type of sugar. This suggests that those particular carbohydrates with which the lectin interacts, or other sugars in close proximity, might be involved in the aggregation process. Since several other aggregation factors either cannot be inactivated or can only be inactivated poorly, the question arises as to whether the appropriate lectins for the functional carbohydrates of those factors have not yet been found, or whether these aggregation factors, in fact, operate via a different type of aggregation mechanism, not involving carbohydrates.
4. Preliminary and unpublished studies by Turner have shown that oligosaccharide pieces can be isolated from the *Microciona prolifera* aggregation factor that turn out to be powerful inhibitors of *Microciona prolifera* reaggregation.

The model developed therefore predicts the presence of a carbohydrate-recognizing component of the *Microciona* aggregation factor. After the execution of a number of procedures that are known to release surface components, the treatment of Ca^{2+}–Mg^{2+} free seawater-dissociated sponge cells with hypotonic NaCl removes some components and makes the cells refractory to the subsequent addition of aggregation factor (figure 5). When the supernatant of that hypotonic treatment was preincubated with such cells that have a defective surface, and the cells were then rinsed, their sensitivity to the addition of aggregation factor was restored (Weinbaum & Burger 1973). The technique for the release of the surface material, which for the time being we will call 'baseplate', still requires several standardizations for each batch of cells and needs improvement. To date, we have very little data on the biochemical characterization of the baseplate, since its functional aspects will have to be worked out first. However, most tests previously carried out confirm the notion that the hypotonic supernatant containing the baseplate has the predicted characteristics (see table 1).

As is the case for most tests on whole and intact cells, they unfortunately include a number of uncertainties. Cell-free model systems can avoid many of them. With that aim, we insolubilized

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the proteins from the hypotonic shock medium by covalently coupling them to solid beads (figure 6, see also Weinbaum & Burger 1973). Such beads aggregate as soon as aggregation factor from the homologous cell is added (table 2). They also attach to mechanically disrupted cells, that still carry aggregation factor, but not, however, to Ca^{2+}–Mg^{2+} free seawater treated cells that therefore were free of functioning aggregation factor. The aggregation of baseplate beads induced by aggregation factor can be inhibited with glucuronic acid (figure 6 and table 2). Inhibition was primarily seen when beads were preincubated with glucuronic acid and not when the aggregation factor alone was preincubated (see table 2). Such experiments promise a quantifiable study of surface interactions between cells by means of *in vitro* systems. It is unlikely that all types of cell surface interactions can be faithfully mimicked with such coated bead systems, because many properties of the cell surface membrane are not included in the bead. For example, the lipophilic core structure of the membrane, furthermore membrane fluidity,

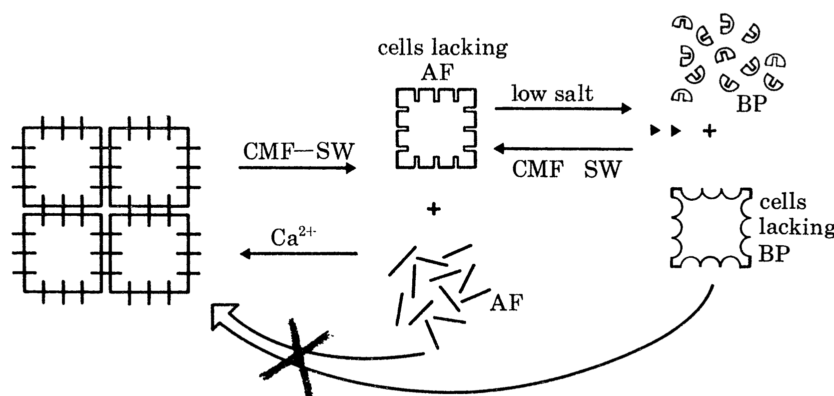

FIGURE 5. Requirement of a membrane-bound baseplate for the specific sorting-out process. Sponge cells dissociated in Ca^{2+}–Mg^{2+} free seawater (CMF-SW) will release aggregation factor (AF) that is required in the presence of calcium (Ca^{2+}) for reaggregation of the cells lacking the aggregation factor. Low salt treatment of such aggregation factor less cells (hypotonic swelling) will release a baseplate or receptor (BP) for the aggregation factor. (From Burger 1974.)

TABLE 1. WHOLE CELL AGGREGATION STUDIES

expt. no.	cell type used	aggregation factor added	baseplate added	glucuronic acid	degree of aggregation
1	CMF	–	–	–	0
2	CMF	+	–	–	4+
3	CMF	second*	–	first*	0
4	CMF+HY	+	–	–	0
5	CMF+HY	–	+	–	0
6	CMF+HY	first†	second†	–	0
7	CMF+HY	second†	first†	–	3+

Where present 0.05 ml *Microciona prolifera* aggregation factor, 0.3 ml baseplate (about 700 µg protein/ml), 5×10^{-2} M neutralized glucuronic acid were used in a final volume of 2 ml seawater. The reaction was started by the addition of another ml containing 5×10^7 *Microciona prolifera* cells/ml. CMF, calcium-magnesium free seawater dissociated *M. prolifera* cells; CMF+HY, the same cells after an additional treatment with a hypotonic NaCl solution. 15 min after mixing the cells at room temperature the samples were scored according to an earlier description (Turner & Burger 1973).

* First reagent was in contact with the cells for 5 min and then only was the reaction started without rinsing in between.

† After preincubation of the cells with the first agent the cells were spun and resuspended in the second reagent. Data partially from Weinbaum & Burger (1973).

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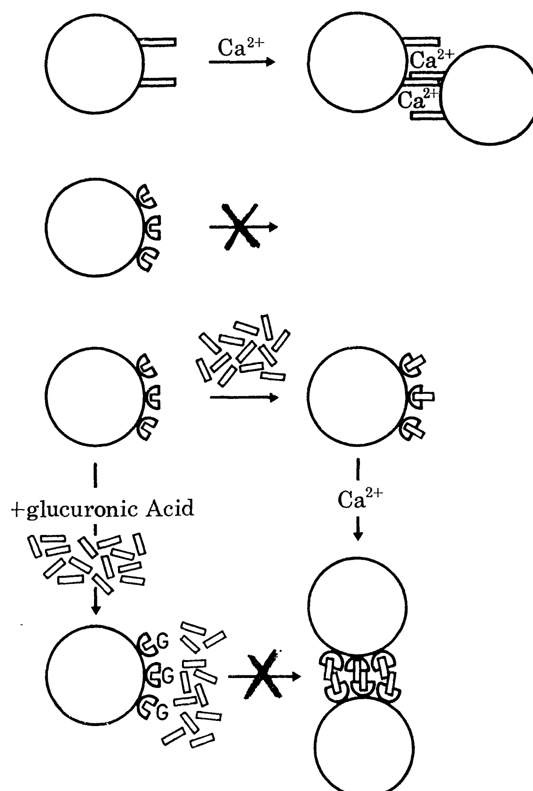

FIGURE 6. Aggregation of factor or baseplate substituted beads. On the first line beads which carry the aggregation factor (bars) are shown to aggregate in the presence of calcium (Ca^{2+}). This unspecific reaggregation could not be inhibited with glucuronic acid. Beads substituted with baseplate (kidney-shaped receptors with groove for aggregation factor) do not aggregate. If, however, aggregation factor (bars) is presented (third line) and then calcium is added, aggregation takes place (third to fourth line on the right). This aggregation could be inhibited by glucuronic acid only (third to fourth line on the left and bottom).

TABLE 2. BEAD AGGREGATION STUDIES WITH IMMOBILIZED AGGREGATION FACTOR AND BASEPLATE

expt. no.	bead type	aggregation factor added	baseplate added	glucuronic acid	degree of aggregation
1	AF	–	–	–	3+
2	AF	–	–	+	3+
3	BP	–	–	–	0
4	BP	+	–	–	4+
5	BP	preincubated	preincubated	–	0
6	BP	second*	–	first*	0
7	BP	first*	–	second*	4+
8	AF+BP	–	–	–	4+

0.05 ml of a suspension of beads substituted with aggregation factor (AF) or baseplate (BP) was added to the 2 ml calcium–magnesium free seawater. Aggregation factor, baseplate and glucuronic acid solution as in table 1. Reaction started with 1.0 ml Ca^{2+}-containing seawater.

* First reagent was in contact with beads for 2 min. Only then was the second added and the degree of aggregation of the beads occurred almost immediately after addition of the calcium-containing seawater and the reactions were scored as described earlier. Data partially from Weinbaum & Burger (1973).

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and in particular, the potential mobility of a glycoprotein within the natural membrane, are quite obviously absent when membrane components are covalently attached to a rigid bead, and consequently such properties cannot be studied in this system.

6. A working model and discussion

Figure 7*a* summarizes our present working model for the reaggregation of *Microciona prolifera* cells. For other cell pairs, as well as for organ-specific cell–cell recognition in vertebrate organisms, we still have to consider inverted polarities where the recognizing moiety lies between the cells and the recognized site at the surface (7*b*), and particularly a model where each surface carries male and female parts, i.e. the antibody-like groove that defines the steric or chemical specificity of the antigen on the same surface complex (7*c*).

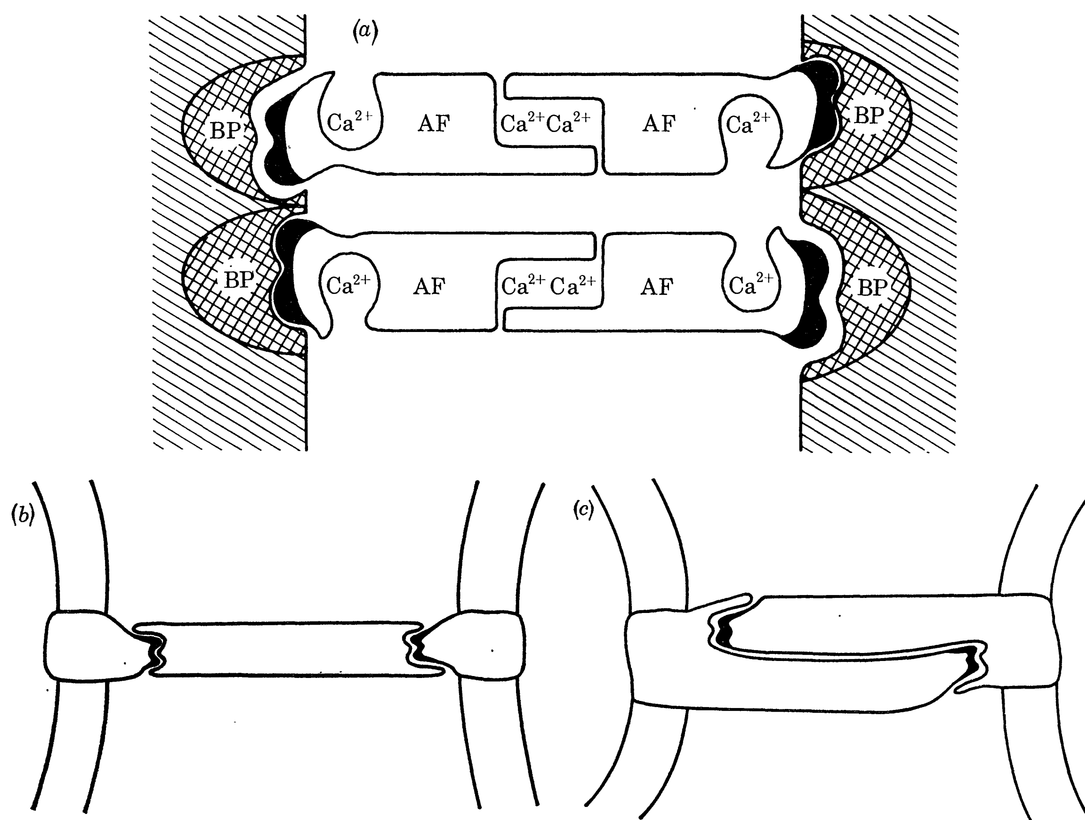

Figure 7. Three possible molecular mechanisms for surface specific cell–cell recognition.

(*a*) Tentative model for the recognition observed in the sponge *Microciona prolifera*. Two macromolecular aggregation factors (AF) are illustrated each consisting of at least two subunits. The black termini at each pole carry the carbohydrates that are recognized by the baseplate (BP) anchored in adjoining cell surfaces. The calcium which keeps the subunits together in this model is required for function of the aggregation factor and is removed by EDTA or EGTA. The calcium at the periphery of the aggregation factor is removed by calcium–magnesium free seawater and is meant to help in stabilizing the aggregation factor at the cell surface. It does not have to be bound to the aggregation factor though. It can fufill such a stabilization function also by being bound to other molecules in the neighbourhood of the aggregation factor (from Weinbaum & Burger 1973).

(*b*) Inversion of the polarity: the recognizing component lies between the cells and the antigenic or recognized component in the surface.

(*c*) Recognizing and recognized component are in the same unit. They are both anchored in close contact or possibly the same cell membrane and not easily extractable.

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Although sponge reaggregation, the biological function of which incidentally is not clear so far, may turn out to be a very fruitful field of study to elaborate some relevant molecular mechanisms for cell–cell recognition in general, we have to be aware that vertebrate cell–cell interaction may be restricted to only one of those types found in invertebrates or may have evolved their own type.

Roseman (1970) has put forward a working hypothesis for recognition between cells based on surface transglycosylases recognizing the oligosaccharide substrate on the opposite cell. This is biologically as well as biochemically an attractive model. Unambiguous evidence for the involvement of this enzyme in morphogenetic recognition and movement is, however, missing for the time being.

Some of the basic questions in the field of morphogenetic migration and reshaping during embryogenesis are still the following: do they take place and are they guided and controlled entirely from within the cell or are they also guided by cell–cell communications? If they are controlled by cell–cell communications, are such communications provided for by intercytoplasmic bridges, as gap junction for instance, or are chemical surface components involved in the recognition process and thereby cell guidance? The next questions, which one will have to ask, are more familiar to the developmental biologist and molecular biologist: how does the information for the distribution of such surface recognition sites over the early embryo take place? How is the formation of such cell–cell recognition systems turned on, and how is it turned off in late embryo and adult cells? Finally, one would like to know how the proper cell distribution is eventually fixed and stabilized. It would be unlikely, if within this catalogue of important questions cell surfaces would not play an important role somewhere, and provide the membrane biochemist with some formidable conceptual and technical problems.

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