Review Letter

FACTORS MEDIATING CELL–CELL RECOGNITION AND ADHESION

Galaptins, a recently discovered class of bridging molecules

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1. Introduction

In view of the importance of cell–cell interactions to an understanding of developmental biology and diseases such as cancer, there is at present considerable interest in the surface molecules by which cells recognise and adhere to one another [1–3]. Here, recent progress is discussed, with particular consideration of the activities and possible functions of a recently discovered class of molecules, for which we propose the name 'galaptins'.

2. Historical perspective

The most popular explanation of cell–cell recognition and adhesion is that the phenomena observed are mediated by specific cell surface ligand molecules. This has been termed the 'specific adhesion hypothesis' [1]. Like other biological recognition systems, cell–cell interactions demand selectivity, speed and reversibility, indicating that non-covalent, high-affinity bonding is involved. This is most easily visualized via configurational complementarity between recognising and recognised agents, the Schloss und Schlüssel (lock and key) analogy of Emil Fischer [4] providing a readily appreciated mechanistic model. The concept that cellular receptors exist for exogenous proteins was first proposed by Paul Ehrlich in the Croonian Lecture to the Royal Society in 1900 [4a]. Ehrlich's remarkably prophetic hypotheses were derived from his studies on toxin and anti-toxin interactions and have culminated in the intensive investigation of the mechanism by which such agents as toxins, drugs, hormones and neurotransmitters are 'recognised' by cellular receptors.

The theory that similar mechanisms are also involved in some instances of cell–cell recognition was championed in the '30s' and '40s' by several scientists, including Weiss, who proposed that an antibody–antigen type of interaction was responsible for cell–cell recognition [5]. The elaboration of this concept was stimulated by the discovery of plant lectins which are multivalent non-immunoglobulin proteins or glycoproteins which bind non-covalently to specific cell surface oligosaccharides and are thus capable of crosslinking cells [6]. Lectins were subsequently discovered in animal tissues also [7] and current evidence indicates that this type of protein–carbohydrate interaction is of primary importance in cell–cell recognition and adhesion systems [3].

3. A classification of recognition and adhesion factors

The molecular mechanisms of factor-mediated cell–cell recognition and adhesion, considered in the simplest terms, must involve molecules anchored in the cell membrane which either:

I. Bind directly to complementary molecules on the apposing cell surface; or
II. Are crosslinked to them by multivalent bridging factors.

Within these two basic mechanisms there are many possible variations, for example:

(i) The membrane-anchored factors may be mirror images of each other or may be of entirely dissimilar structure;
(ii) The functional bridging molecules may be either multivalent or multimers of univalent subunits and they may bridge between cells or between endogenous membrane anchored molecules thereby organising recognition sites which bind to the apposed cell surface.

Table 1
Factors mediating intercellular recognition and adhesion

Factor	Nature	Subunit M_r (no./molecule)	Delipidising extraction used?
I. Intrinsic membrane factors			
Contact sites A [22] Appear on surface of slime mould *D. discoideum* during aggregation. Anti-contact site A Fab blocks adhesion	Glycoprotein	70 000 –80 000 (1)	Yes
71 000 M_r antigen [55] Present on vegetative cells of *P. pallidum* but strongly synthesized during aggregation. Anti-71 000 M_r antigen Fab inhibits aggregation	Glycoprotein	71 000 (1)	Yes
Cognin [30–33,37] Synthesized during retinal development in chick embryo and induces enhanced reaggregation in vitro	Glycoprotein	50 000 (1)	Yes
CAM [34–36,38,39] Present on retinal cell plasma membranes during chick embryonic development and probably mediates side-to-side adhesion between neurites during embryogenesis. Anti-CAM Fab disrupts histogenesis of embryonic retina in organ culture	?	140 000 (2–4)	Yes
Hepatic binding protein [56,57] Binds circulating desialated erythrocytes and glycoproteins in rat and rabbit liver. Galactose specific	Glycoprotein	40 000 48 000 (12)	Yes
Gamete receptors in *Fucus serratus* (brown algae) [58] Sperm receptor on egg Egg receptor on sperm Recognition is species specific and involves fucose and mannose residues on sperm receptor	 Glycoprotein Glycoprotein	 30 000 (?) 60 000 (?)	 Yes Yes
ZP3 Mammalian sperm receptor [59] One of the 3 glycoproteins of mouse zona pellucida. Modified on fertilization	Glycoprotein	83 000 (?)	Yes
Strain 21 factor [12] Mediates aggregation with compatible yeast (strain 5) of *Hansenula wingei* during conjugation. The monovalent factor is released from the cell wall by trypsin or zymolase and neutralizes strain 5 factor	Glycoprotein	27 000 (?)	No
II. Extrinsic 'bridging' factors			
(a) *Large multivalent proteoglycans*			
Aggregation factor [13–21] Released when marine sponges are disaggregated in Ca^{2+}-free sea-water. Induces species-specific reaggregation *Microciona parthena* [15] *Geodia cydonium* [21]	 Proteoglycan Protein/carbohydrate/lipid	 21×10^6 (?) 103×10^6 (?)	 No No
Strain 5 factor [11,60] Mediates aggregation with compatible yeast (strain 21) of *Hansenula wingei* during conjugation. The multivalent factor is released from the cell wall by protease treatment and agglutinates strain 21 cells	Proteoglycan	9.6×10^5 (?)	No

(*continued*)

Table 1 (continued)

Factor	Nature	Subunit M_r (no./molecule)		Delipidising extraction used?
(b) *Protein lectins*				
(i) *Heterogenous*				
Discoidin [66,67] *N*-acetyl galactosamine specific. Two isolectins identified	Protein	29 500 26 500	(4)	No
Pallidin [26] β-Galactoside-specific. Three isolectins identified These lectins appear on the cell surface of slime mould amoebae during the aggregation phase of the life cycle	Protein	26 500 26 000 25 000	(multimers of 3)	No
Bindin [61] Major component of the tip (acrosome vesicle) of the sea urchin sperm head. Binds to specific egg surface glycoprotein oligosaccharides of the homologous species. Isolated with the aid of detergents but appears to be in a granular, nonmembranous form in vivo. Insoluble and particulate in vitro in the absence of denaturing agents	Protein	30 500	(?)	Yes
(ii) *Galaptins*				
Low molecular weight, β-galactoside-specific, animal lectins				
Chick tissue lectin [40,41,44,62] Lectins in embryonic muscle, brain and liver are identical by SDS–PAGE, isoelectric focusing and immunoassay	Protein	15 000	(2)	No
Calf tissue lectin [42,63] Lectins in liver, spleen, thymus and heart are identical by SDS–PAGE, isoelectric focusing and immunoassay	Protein	12 000	(2)	No
Rat myoblast lectin [40,51,64] Specific activity increases commensurate with myoblast fusion and subsequently declines	(?)	13 000	(?)	No
Rat lung lectin [45,65] Activity in neonatal lung correlates with synaptogenesis	(?)	13 500	(2–4)	No
EDA, erythroid developmental agglutinin [48] Probably responsible for mutual erythroblast adhesion observed during erythropoiesis in rabbit bone marrow. A bridging role is indicated by the fact that anti-EDA Fab blocks cell adhesion in vitro	Protein	13 000	(?)	No

Nevertheless, those molecules which have so far been characterized and experimentally demonstrated to be involved in intercellular recognition and adhesion fall naturally into the two categories delineated, that is they are either membrane-anchored factors or extracellular factors which, it is suggested, bridge between cells or receptors. The classification of well-characterized factors is shown in table 1.

The anchored molecules appear to be intrinsic membrane components as described by Singer and Nicolson in the fluid mosaic model of membrane structure [8] because, like other integral membrane proteins such as glycophorin, the HLA antigens and G protein of vesicular stomatitis virus, they are large glycoproteins extracted from the membrane only by delipidising agents such as detergents and organic sol-

vents. It seems probable that intrinsic glycolipid receptors will also be identified for there is strong evidence suggesting that the ganglioside G_{M1} is a receptor for cholera toxin [9] and that a specific acidic glycolipid is the receptor for macrophage migration inhibition factor (MIF) [10].

Conversely the 'bridging' molecules appear to be extrinsic to the membrane and non-covalently bound to intrinsic membrane receptors. Most 'bridging' factors appear to bind via protein–carbohydrate interactions, analogous to plant lectins, and therefore can, in many cases, be extracted from the cell surface by aqueous media containing appropriate sugars. In terms of size and molecular structure the extrinsic factors fall naturally into two subgroups: the large multivalent proteoglycans and the protein lectins. The latter can be subdivided into a heterogenous group of factors and the low molecular weight (or M_r) β-galactoside specific, animal lectins, termed here galaptins.

Our current understanding of the means by which these various factors mediate cell–cell recognition and adhesion is illustrated here by the discussion of recent progress in some experimental systems.

4. Mating-type interactions in yeast

Sexual reproduction in ascomycetous yeasts involves the fusion of two unicellular organisms of opposite mating type. The cell surface molecules which mediate sexual aggregation between the compatible strains, 5 and 21, of *Hansenula wingei* constitute the only recognition and adhesion system where both complementary factors have been well characterized. The strain 5 factor is a large multivalent proteoglycan (85% carbohydrate, almost all of which is mannose, 10% protein, 5% phosphate) released from the cell wall by protease digestion. It agglutinates strain 21 cells and is heat-stable but dissociated by reducing agents into several monovalent recognition sites, which bind to strain 21 cells, and a glycoprotein core [11]. It must be remembered that this factor is anchored in the yeast cell wall and therefore not strictly comparable to other cell adhesion factors intrinsic to plasma membranes. For this reason, and because of the extensive similarity to sponge aggregation factors (discussed below) strain 5 factor is classified as an extrinsic 'bridging' molecule (table 1) although it can only be solubilized by enzyme treatment.

The complementary factor in the cell wall of strain 21 cells is released by trypsinization and is a heat-labile acidic glycoprotein which neutralizes the aggregation activity of the 5 factor but does not agglutinate strain 5 cells [12]. This factor is classified as an intrinsic factor because of its molecular nature and monovalent activity. Isolated 5 factor and 21 factor form complexes in vitro but the molecular mechanism by which these factors recognise and adhere to one another remains undetermined.

The agglutination factors from two other pairs of compatible strains have been reported to show similar properties, that is, one cell type of each pair is heat-stable and inactivated by reducing agents while the complementary cell type is heat-labile and insensitive to reducing agents [12].

5. Marine sponges

The first experimental demonstration of species-specific intercellular recognition and adhesion in multicellular organisms was reported by Wilson, who in a series of experiments between 1907 and 1911 investigated the selective reassociation of different species of marine sponge, after their mechanical disaggregation into single cells. Fifty-two years later Humphreys [13] and Moscona [14] dissociated sponges in Ca^{2+}- and Mg^{2+}-free sea-water and showed that reassociation depended on the presence of a soluble 'aggregation factor' released into the medium during this procedure. Subsequent work by these and other investigators has identified several species-specific aggregation factors.

The factors purified from *Microciona prolifera* and *Microciona parthena* are large proteoglycan molecules composed of approximately equal amounts of protein and carbohydrate. Under the electron microscope they both show an unusual 'sun-burst' configuration of a circular core surrounded by numerous radiating arms [15,16]. The aggregation activity is Ca^{2+}-dependent and may involve binding via glucuronic acid residues on the aggregation factor [17]. Burger and his associates have reconstituted an aggregation system in vitro by immobilising purified factor and fractions containing the probable receptor on Sepharose beads [18]. The results of a series of recombination and inhibition experiments correlate with all the characteristics of reaggregation in vivo and strongly support the postulate that the factor purified is responsible for the in vivo species-specific adhesion.

Jumblatt et al. [19] have recently shown that the Ca^{2+}-dependent process is the self association of aggregation factor, both in the agglutination of sponge cells and of derivatised Sepharose beads. Two other important observations are reported in [19].

(i) The aggregation of factor-derivatised beads is promoted by the addition of free factor which indicates that crosslinking is more effectively mediated by aggregation factor complexes than by bimolecular bridging interactions.

(ii) Whilst the in vitro aggregation of cells requires only 400 factor molecules/cell, ~28 000 molecules/cell are found in vivo.

Since it has been reported that preparations of aggregation factor tend to form gels at elevated $[Ca^{2+}]$ it seems logical to suppose that these factors form extracellular multimolecular aggregates in vivo analagous to the proteoglycan aggregates in mammalian cartilage.

The reaggregation of *Geodia cydonium* cells dissociated in Ca^{2+}- and Mg^{2+}-free sea-water has been divided into several stages [20]. After an initial association into small clumps, the formation of larger aggregates is initiated by a species specific aggregation factor distinct from, though apparently similar to, those of *Microciona* sponges. Müller et al. have reported that the *G. cydonium* aggregation factor is associated with a series of glycosyltransferase enzymes in a large circular molecule of 1.3×10^8 M_r, composed of 74% protein, 10% carbohydrate and 5% lipid. The core structure of this complex, revealed by SDS treatment of Ca^{2+}-aggregated multimers, also appears as a sunburst configuration in electron micrographs [21].

6. Cellular slime moulds

During the vegetative growth phase the cellular slime moulds exist as a population of amoebae but in adverse growth conditions these cells aggregate to form a multicellular psuedoplasmodium, or slug, which produces a fruiting body to distribute a new generation of vegetative cells as spores. When vegetative cells begin to form the psuedoplasmodium they are termed 'aggregation competent' and much research has centred on the molecular mechanism of this change.

In *Dictyostelium discoideum* monovalent Fab fragments from antisera raised against the cell surface antigens of aggregation competent cells were found to inhibit the characteristic end-to-end adhesion of elongated amoebae seen during slug formation. The active component binds to a minor cell surface glycoprotein, called contact site A [22]. Similar sites are found on growth phase cells, mediating side-to-side adhesion and have been called contact sites B. Growth phase and developmentally regulated target sites for adhesion blocking Fab fragments have also been identified in *Polysphondylium pallidum*.

The adhesion mechanisms operating in growth phase cells do not appear to be species specific in that mixed aggregates form in gently shaken suspensions of vegetative *D. discoideum* and *P. pallidum* cells. When the cells attain aggregation competence, however, they separate out into largely monospecific aggregates indicating that the developmentally regulated cell recognition mechanism involving contact sites A is highly selective and thereby species specific [24]. It has not yet been determined whether contact sites A act via a reciprocal binding in vivo or if other factors are involved.

This picture of the molecular mechanisms of cell–cell recognition and adhesion in the slime moulds is complicated by the presence, previously reported, of another recognition system which involves extrinsic 'bridging' factors. In both *D. discoideum* and *P. pallidum* species-specific protein lectins, called discoidin [25] and pallidin [26], respectively, appear on the surface of vegetative cells as they attain aggregation competence. Whilst the postulate that these proteins mediate cell aggregation via a 'bridging' mechanism is not supported by the inability to block the aggregation of competent cells with anti-lectin antibodies (in direct contrast to the results with contact sites A and B) [23,24,27], the recent discovery of an aggregation-defective mutant of *D. discoideum* which lacks functional discoidin but appears to bear active receptors [28] suggests that these proteins do play an essential role in cellular recognition and adhesion. An obvious unifying hypothesis is that contact sites A constitute the membrane-anchored receptors for the 'bridging' lectins. However, it has been reported that preincubation of a crude discoidin fraction with a large excess of purified contact sites A does not inhibit the subsequent agglutination of sheep erythrocytes by discoidin [29]. Therefore the possibility that these molecules participate independently in two, as yet incompletely elucidated mechanisms of recognition and adhesion must be contemplated.

7. Chick embryonic tissues

Considerable research effort has been expended in investigation of the mechanisms of specific intercellular recognition and adhesion in embryonic tissues. The foundation for the specific adhesion hypothesis in embryological systems was laid down by Moscona et al. in a series of experiments which they began in the '50s'. Their investigations have ultimately centred on chick embryonic neural retina tissue, where they have identified a cell surface glycoprotein which enhances the reassociation of trypsin dispersed tissue in vitro [30,31]. The presence of similar factors in chick embryonic cerebrum and spinal cord has been demonstrated also [32] and Moscona has suggested that these molecules be termed 'cognins', to indicate their postulated role in the mechanisms of mutual recognition and morphogenetic association of embryonic cells. Edelman et al., utilising adhesion blocking monovalent Fab fragments to isolate cell surface molecules involved in adhesion, have identified in chick neural retina a cell adhesion molecule (CAM) which also seems to be involved in the reassociation of retinal cells in culture [34–36].

The different experiments performed by these two groups make it impossible to compare directly or to assess the molecular similarity of these two membrane components. However, certain similarities and differences are notable. Both molecules reappear on the surface of trypsinized retinal cells in culture and are also found in conditioned media. Antisera to the proteins shows that their appearance at the cell surface is developmentally regulated and correlates with periods of maximum morphogenesis within the embryonic retina. Cognin, however, is a glycoprotein of M_r 50 000, both on sodium dodecylsulphate (SDS)–polyacrylamide gel electrophoresis and in native solution, whereas CAM has app. M_r 140 000 on SDS–polyacrylamide gel electrophoresis. The carbohydrate content of CAM has not been reported. The activity of these two molecules is assayed by quite different techniques. The addition of purified cognin has been shown to specifically promote the reaggregation and morphogenetic reassociation of freshly trypsinized cells in rotating suspension culture over 24 h whilst CAM activity is inferred because, after retinal cells have been cultured to recover from trypsinization, the addition of specific anti-CAM Fab fragments inhibits reaggregation during a 20 min incubation. Anti-cognin antisera has been shown to interfere with retinal reassociation when cells which have recovered from trypsinization are incubated with antisera for 1 h, rinsed and cultured normally for 24 h [37]. However, the long incubation time and the use of divalent antibodies rather than monovalent Fab fragments in this experiment makes the results difficult to interpret.

Consideration of the assay systems by which these factors were detected suggests that CAM might mediate initial, short-term interactions and cognin secondary, long-term associations. However, the application of anti-CAM Fab to retinal cells cultured through to neurite formation [36], to the outgrowth of neurites from spinal ganglion cultures [38] and to isolated embryonic retina in organ culture [39] results in a disruption of tissue development and a decrease in regions of cell–cell contact, suggesting that one of the major functions of CAM is to mediate side-to-side adhesion between neurites during embryogenesis. The effect of anti-cognin Fab fragments on such long-term cultures has not been reported.

8. Galaptins

'Galaptin' is the generic term which we propose for the low M_r, protein, β-galactoside-specific, animal lectins which have recently been isolated from a number of developmental and adult tissues. The word is derived from their saccharide specificity and the Greek 'hapto'- to touch or to join. They were first identified in the electric organ of the electric eel [7] and subsequently in chick embryonic tissues [41].

Galaptins may be extracted by washing single cell suspensions or tissue homogenates with lactose-containing media and purified by various affinity chromatography techniques. They have M_r 13 000–15 000 on SDS–polyacrylamide gel electrophoresis, though some appear dimeric on gel filtration, and they demonstrate a divalent cation-independent, β-galactoside-specific haemagglutination activity. Galaptins from embryonic chick brain, muscle and liver appear essentially identical by SDS–polyacrylamide gel electrophoresis, isoelectric focusing and immunoassay [41], as do those isolated from calf liver, spleen, thymus and heart [42]. The chicken and calf lectins show only weak immunological crossreactivity [42], indicating a degree of species specificity, but antigenic crossreaction between galaptins of bovine and human heart and bovine heart and human and rhesus monkey skeletal muscle has been reported [43].

In chick embryo [44] and neonatal rat lung [45] the galaptin concentration in the tissue is developmentally regulated and is maximal at times of extensive tissue organisation. This observation and the identification of galaptins at the cell surface in some tissues [46–48] supports the postulate that these proteins might be involved in intercellular recognition and adhesion. Their immunologic crossreactivity might be considered to argue against a tissue-specific activity but it is not unreasonable to suppose that molecules with similar, though specific, functions would bear some common and some unique determinants and a highly conserved protein structure could be taken to indicate a crucial role within the organism.

A developmental system in which much evidence to support this hypothesis has been obtained is that of erythroid development in adult mammalian bone marrow, where differentiating erythroblasts are clustered closely together in 'erythroblastic islands' around a central macrophage nurse cell. Erythroid developmental agglutinin (EDA), a typical galaptin, has been purified from adult rabbit bone marrow and shows a developmentally regulated and tissue-specific activity in vivo and in vitro [48]. Cell surface EDA is detected only on erythroblasts in bone marrow and EDA specifically agglutinates erythroblasts in vitro. Moreover, both the cell surface distribution of EDA and the susceptibility of washed erythroblasts to agglutination by EDA decline on erythroid maturation, concomitant with the release of reticulocytes from the erythroblastic island observed in vivo. These observations suggest that EDA might mediate inter-erythroblast associations in vivo.

This view is supported by the ability of EDA to crosslink inert glutaraldehyde-fixed, trypsinized erythrocytes and by the inhibition of EDA-mediated erythroblast agglutination in vitro by anti-EDA Fab fragments. These results indicate that EDA probably acts as an extrinsic cell–cell 'bridging' factor. The apparent structural similarities between galaptins suggest that all these factors will possess the ability to act as 'bridging' molecules between cells and they could play a particularly useful role in establishing initial short term and readily reversible cell–cell contact in developmental systems. In erythropoiesis, EDA-mediated inter-erythroblast adhesion operates at an early stage in tissue development, but cohesion is reversed in this system when the reticulocyte is released from the erythroblastic island prior to entering the circulation. In other tissues such links might be superseded by more permanent binding mechanisms.

It seems that this cannot be the only role of galaptins in vivo, however, because their presence in several adult tissues has recently been discovered ([43,49, 50], F. L. H., unpublished). In these tissues, as in certain embryonic tissues, they seem to be primarily located intracellularly [42,49,51], though in chicken pancreas they are located in the extracellular spaces surrounding the pancreatic acini [49]. Galaptins may be considered to be primarily specific carbohydrate-binding molecules and their role both at the cell surface and within the cell could be to bind and transport glycoproteins. The distribution of a small number of recognition molecules at the cell surface and in greater proportion within the cell is reminiscent of the cellular distribution of hepatic binding protein, which mediates the internalization of circulating desialated glycoproteins and is then recycled [52]. Similar receptors capable of recognising extracellular hormones and growth factors may be essential both in adult tissues and in developmental systems and galaptins would be suitable molecules for such functions. The report that myotube formation in culture is inhibited only by prolonged culture with galaptin-inhibitory sugars is consistent with this hypothesis [51].

It could be that in fulfilling these functions, or the many other functions suggested for animal lectins (e.g., [53]) these molecules are exhibiting a protein–carbohydrate recognition and interaction analagous to the protein–protein recognition processes long accepted as a basic biochemical phenomena and their ability to self-associate and thereby crosslink cells may be entirely fortuitous. The 'lectin' activity associated with such molecules as glycophorin [54] might be taken to support this view.

9. Conclusion

Factors which mediate cell–cell recognition and adhesion have been identified and characterised in a number of experimental systems and appear to be of two main types: (I) intrinsic membrane factors or (II) extrinsic 'bridging' factors. We feel that this expanding field of research can be clarified by a classification of adhesion factors based on these two fundamental characteristics, as presented in table 1.

Galaptins is the generic term which we propose for

the low M_r, protein, β-galactoside-specific, animal lectins described in a number of developmental and adult tissues. Recent work has suggested that these lectins may mediate adhesion via a cell–cell 'bridging' mechanism in some developmental systems.

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