Cell-surface discoidin in aggregating cells of *Dictyostelium discoideum*

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Both discoidin I and discoidin II have been detected on the surface of aggregating (10h developmental stage) cells of *Dictyostelium discoideum* NC4 by radioiodination of the cell-surface followed by immunoprecipitation and sodium dodecyl sulphate/polyacrylamide-gel-electrophoretic analysis. Approx. 92% of cell-surface discoidin I and 72% of cell-surface discoidin II can be eluted with 0.5 M-galactose, showing that most of each endogenous lectin is not present as integral membrane protein but rather is bound to cell-surface discoidin receptors. Two-dimensional polyacrylamide-gel-electrophoretic analysis of discoidin I suggests that the native tetramer may be a hetero-multimer composed of both Ia and Ib subunits. Cell-surface discoidin I also contains both types of subunit, but it is not clear whether both subunits have corresponding cell-surface receptors.

Following the removal of nutrients, amoebae of the cellular slime mould *Dictyostelium discoideum* aggregate to form multicellular complexes which then undergo further development to form fruiting bodies. Two endogenous lectins with a binding specificity for sugars structurally related to galactose, discoidin I and discoidin II, are accumulated by the cells during aggregation (Frazier *et al.*, 1975) and have been suggested to have a role in cell cohesion (Frazier *et al.*, 1975; Reitherman *et al.*, 1975; Ray *et al.*, 1979; Shinnick & Lerner, 1980). Despite the early demonstration that discoidin accumulates on the cell surface (Chang *et al.*, 1975; Siu *et al.*, 1976) as well as inside the cells during aggregation, these studies did not determine whether both discoidin I and discoidin II are cell-surface-located. The mode of attachment of cell-surface discoidin also needs clarification. In a recent study of cell-surface receptors for the discoidins using a lectin binding assay (Madley & Hames, 1981), we found larger numbers of discoidin receptors than had been detected by other workers (Bartles & Frazier, 1980). Unlike those in previous studies, the cells used in our experiments had been washed extensively with galactose before fixation and therefore we argued that the increased numbers of receptors observed may, in part, be due to the elution by galactose of endogenous discoidin bound to, and thereby mask-

ing, cell-surface receptors for this lectin. If correct, this would imply that a significant proportion of cell-surface discoidin is not present as integral membrane protein but attached only via binding to the carbohydrate moiety of a cell-surface receptor.

In the study reported here we demonstrate that both discoidin I and discoidin II are represented on the surface of aggregating *D. discoideum* cells. In agreement with our earlier suggestion, at least 92% of cell-surface discoidin I is bound via galactose-sensitive bonds and hence presumably bound to cell-surface receptor rather than being an integral membrane protein. Similarly, at least 72% of cell-surface discoidin II can be eluted with galactose. Our studies also show that both recently discovered subunits of discoidin I, namely Ia and Ib, are present in cell-surface discoidin but it is not clear whether only one or both subunits are responsible for the cell-surface binding.

Experimental

Radioiodination of *D. discoideum* cell-surface

D. discoideum NC4 amoebae were grown and dispensed on Millipore filters, for development to occur, as described previously (Madley & Hames, 1981). Those cells due to be radioiodinated to detect total cell-surface discoidin were harvested at the 10h (aggregated) stage of development in 17.5 mM sodium/potassium phosphate buffer, pH 6.5 and then washed twice by centrifugation in this buffer. After resuspension at 10^8 cells/ml in this buffer, the cells were radioiodinated essentially by the method

Abbreviations used: IgG, immunoglobulin G; SDS, sodium dodecyl sulphate.

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of Siu *et al.* (1976). Typically, the reaction mixture comprised 1 ml of cell suspension, 10 μ l of lactoperoxidase (Boehringer Mannheim), 2 mCi of carrier-free Na¹²⁵I (Amersham International Ltd.), all in 17.5 mM-sodium/potassium phosphate buffer, pH 6.5. The reaction was started by the addition of 5 μ l of H₂O₂ (final concn. 17 μ M). After 2 min at room temperature, the cells were recovered by centrifugation, washed twice with 17.5 mM-sodium/potassium phosphate buffer, pH 6.5, and resuspended at 10⁷ cells/ml in 0.14 M-NaCl/0.01 M-sodium/potassium phosphate buffer, pH 7.0. Portions (1 ml) were centrifuged to sediment the cells, which were then frozen in liquid N₂ and stored at -70°C. Another batch of the same cells was harvested from Millipore filters at the same (10 h) stage of development and radioiodinated by using the procedures described above, but in this case all buffers used for cell harvesting, radioiodination and post-radioiodination washing contained 0.5 M-galactose to elute any endogenous discoidin bound to cell-surface receptors.

Preparation of anti-discoidin IgG and non-immune IgG

Antibodies were raised against a mixture of discoidin I and II purified by affinity chromatography from *D. discoideum* AX3 as described by Frazier *et al.* (1975). New Zealand male rabbits received initial injections of 1 mg of discoidin emulsified in Freund's complete adjuvant, followed by a second injection of 1 mg of discoidin in Freund's incomplete adjuvant 4 weeks later, both injections being delivered at several sites subcutaneously. Bleeding was carried out 1 and 2 weeks after the second injection and at similar time periods after booster injections. After inactivation of complement by heating at 56°C for 30 min, a crude IgG preparation was obtained by precipitation with (NH₄)₂SO₄ to 40% saturation, followed by dissolution in 0.14 M-NaCl/0.01 M-sodium/potassium phosphate buffer, pH 7.0, and dialysis against this buffer. The dialysed immunoglobulin was then passed down a column of discoidin-Sepharose 4B (prepared by cross-linking a mixture of discoidin I and II to Sepharose 4B using CNBr) which had been pre-equilibrated in the above dialysis buffer. Unbound immunoglobulin was discarded and then bound anti-discoidin IgG was eluted with 0.5 M-acetic acid. After neutralization with 2.5 M-NH₃, the IgG was concentrated by precipitation with (NH₄)₂SO₄ and then dialysed as above. The titre of the purified anti-discoidin IgG was determined by immunoprecipitation of known amounts of radioiodinated discoidin I or radioiodinated discoidin II.

Non-immune rabbit IgG was purified by essentially the same protocol as that described above, but

an affinity matrix of goat anti-rabbit IgG-Sepharose 4B was used.

Immunoprecipitation of radioiodinated cell-surface discoidin

Cell pellets were thawed after addition of 2 ml of 1 mM-phenylmethylsulphonyl fluoride/0.5 M-galactose/0.5% Nonidet P40/0.14 M-NaCl/0.01 M-sodium/potassium phosphate buffer, pH 7.0, and then sonicated for a total of 20 s in short bursts. After centrifugation at 100 000 *g* for 1 h, the supernatant was divided into two equal portions and 2.5 μ g of affinity-purified discoidin I and II mixture (Madley & Hames, 1981) was added as carrier. Then 87 μ g of affinity-purified anti-discoidin IgG, or an equivalent amount of non-immune rabbit IgG, was added and immunoprecipitation was allowed to occur at 4°C for 4 h. The immunoprecipitates were collected by centrifugation at 15 000 *g* for 10 min and each was washed twice with 200 μ l of 1% Triton X-100/1% (w/v) sodium deoxycholate/0.15 M-NaCl. The final pellets were resuspended in 25 μ l of 1% (w/v) SDS/5% (v/v) β -mercaptoethanol/2% (v/v) Ampholines [a 4:1 (v/v) mixture of pH 5-8 and pH 3.5-10 LKB Ampholines]. For one-dimensional polyacrylamide-gel electrophoresis, 3 μ l of 0.5 M-Tris/HCl, pH 6.5, and 3 μ l of glycerol was added to a 25 μ l portion, whereas for two-dimensional polyacrylamide-gel electrophoresis, samples were saturated with urea and 3 μ l of 20% Nonidet P40 was added before loading.

Binding of radioiodinated discoidin I to D. discoideum cells

Radioiodinated discoidin I was prepared as described previously (Madley & Hames, 1981). *D. discoideum* NC4 cells were harvested at the 10 h stage of development in buffer containing 0.5 M-galactose (to elute any endogenous cell-surface discoidin), fixed with glutaraldehyde, and then incubated with purified discoidin I radioiodinated *in vitro*. After the incubations the cells were washed free of unbound discoidin and then bound discoidin I was eluted with galactose. These procedures have all been described in detail previously (Madley & Hames, 1981). Eluted discoidin I was concentrated by precipitation with trichloroacetic acid, using calf thymus DNA as carrier, dissolved in lysis buffer (9.5 M-urea/2% Nonidet P40/2% Ampholines/5% β -mercaptoethanol) and analysed by dimensional polyacrylamide-gel electrophoresis.

Polyacrylamide-gel electrophoresis

One-dimensional polyacrylamide-gel electrophoresis was carried out using the SDS/discontinuous buffer system of Laemmli (1970) with 6-18% linear-gradient polyacrylamide slab gels. Two-dimensional polyacrylamide-gel electrophoresis of

denatured immunoprecipitated cell-surface discoidin or denatured discoidin radioiodinated *in vitro* was essentially as described by O'Farrell (1975), with 0.4% pH 3.5–10 and 1.6% pH 5–8 Ampholines in the first-dimensional gel, and a 6–18% linear gradient polyacrylamide slab gel as the second-dimensional gel. Two-dimensional polyacrylamide gel analysis of native discoidin radioiodinated *in vitro* was carried out as described above, but with the following modifications. In the first-dimensional electrofocusing gel, urea was omitted from all the buffers used. Three gels were run in parallel, two of which were used for analysis on second-dimensional gels as described below. The third gel was used to check the final pH gradient and to determine the pI of the native discoidin. After electrofocusing, one gel containing native discoidin I was sealed into position, using agarose, on top of a 6–18%-polyacrylamide linear-gradient slab gel prepared as for the usual second-dimensional analysis, but with the resolving-gel buffer supplemented with 7.6 M-urea and the stacking-gel buffer supplemented with 7.6 M-urea and 1 mM-dithiothreitol. The second electrofocusing gel containing native discoidin I was similarly sealed in position on top of an identical 6–18%-polyacrylamide linear-gradient slab gel, except that the resolving gel and the stacking gel both contained 0.3 M-galactose in addition to 7.6 M-urea and 7.6 M-urea/1 mM-dithiothreitol respectively, the electrophoresis buffer also contained 0.3 M-galactose, and the agarose used to seal the electrofocusing gel in position contained 0.5 M-galactose.

Sucrose-gradient sedimentation analysis of discoidin

Confirmation of the oligomeric state of discoidin I was obtained by sucrose-density-gradient centrifugation as described by Bartles & Frazier (1980), who used 5–20% (w/v) sucrose gradients either in 0.075 M-NaCl/0.075 M-sodium/potassium phosphate buffer, pH 7.2, or in this buffer supplemented with 2% Nonidet P40 and 2% Ampholines. Gradients were loaded with 100 μ l of 125 I-labelled discoidin I or 100 μ l of concanavalin A (25 mg/ml) or bovine serum albumin (25 mg/ml) in 0.075 M-NaCl/0.075 M-sodium/potassium phosphate buffer, pH 7.2, or in this buffer supplemented with 2% Nonidet P40 and 2% Ampholines. After centrifugation (165 000 g for 14 h at 4°C) the gradients were fractionated and the position of the 125 I-labelled discoidin I was determined by counting the fractions in an Intertechnique SL-3000 gamma counter. The positions of concanavalin A and bovine serum albumin markers in the gradients lacking Nonidet P40 were determined by A_{280} measurement or, in the gradients containing Nonidet P40, by assaying the protein content of the fractions (Schaffner & Weissman, 1973).

Results

Cell-surface discoidin I and II elutable with galactose

In order to examine the nature of the attachment of discoidin to the cell-surface, aggregating (10h) *D. discoideum* NC4 cells were harvested and washed extensively with 17.5 mM-sodium/potassium phosphate buffer, pH 6.5, or with the same buffer but containing 0.5 M-galactose, before radioiodination of exposed cell-surface proteins by the lactoperoxidase procedure (Siu *et al.*, 1976). Radioiodinated cell-surface-located discoidin was then immunoprecipitated by using specific anti-discoidin IgG and analysed by SDS/polyacrylamide-gel electrophoresis on a 6–18% linear-gradient slab gel. Fig. 1(a) shows the Coomassie Blue-stained gel pattern and indicates that the only polypeptides present in each immunoprecipitate, apart from the IgG heavy and light chains, are discoidin I and discoidin II (Fig. 1a, lanes 2 and 3). Most of the protein in the stained discoidin bands represents discoidin carrier added before the immunoprecipitation. Fig. 1(a), lanes 4 and 5, represent control incubations containing non-immune affinity purified IgG instead of anti-discoidin IgG. Fig. 1(b) is an autoradiograph of the Coomassie Blue-stained gel in Fig. 1(a) and shows that both discoidin I and discoidin II are present on the surface of aggregating *D. discoideum* NC4 cells

Fig. 1. Immunoprecipitation of cell-surface radioiodinated discoidin

(a) Coomassie Blue-stained gel of authentic unlabelled discoidin II (lane 1), immunoprecipitated cell-surface-radioiodinated discoidin from *D. discoideum* cells (10h developmental stage) pre-washed with 0.5 M-galactose (lane 2) and immunoprecipitated cell-surface-radioiodinated discoidin from *D. discoideum* cells (10h developmental stage) harvested in the absence of galactose (lane 3). Lanes 4 and 5 represent control incubations which contained the same samples as lanes 2 and 3 respectively, but with non-immune IgG in place of anti-discoidin IgG. Un-numbered lanes contained trace amounts of marker polypeptides of known molecular weight. (b) Autoradiograph of lanes 2–5 of the gel shown in (a).

Fig. 2. Densitometric scan of radioiodinated cell-surface discoidin

The lower and upper curves are the scans of lanes 2 and 3 of Fig. 1(b) respectively. Discoidin I and II are indicated by arrows.

in a form which is available for lactoperoxidase labelling (lane 3). The amount of cell-surface radioiodinated discoidin I we find is in significant excess over cell-surface radioiodinated discoidin II. Whether this reflects the relative amounts of discoidin I and II present on the cell surface or the differential availability of cell-surface discoidin I and II to lactoperoxidase labelling is uncertain at this time. When the cells are washed with galactose before radioiodination, most labelled discoidin I and II is removed from the cell surface (Fig. 1b, lane 2). This result is not an artefact of the immunoprecipitation procedure, since Fig. 1(a), lanes 2 and 3, demonstrate that immunoprecipitation of the discoidins is equally effective in both cases. Densitometric scanning of the autoradiographic images (Fig. 2) indicates that 92% of discoidin I and 72% of discoidin II on the cell surface of aggregating *D. discoideum* cells can be eluted with galactose.

Both discoidin Ia and discoidin Ib are cell-surface-located

As expected, two-dimensional polyacrylamide-gel

Fig. 3. Two-dimensional polyacrylamide-gel electrophoresis of cell-surface radioiodinated discoidin (a) and (b) are Coomassie Blue-stained gels of immunoprecipitated cell-surface-radioiodinated discoidin from *D. discoideum* cells (10h developmental stage) prepared in the absence or presence of 0.5M-galactose respectively. Discoidin Ia, Ib and II subunits are indicated. The other, less intensely stained spots are immunoglobulin, whereas the intensely stained area at the left bottom of each gel represents residual Ampholines. (c) and (d) are autoradiographs of the gels shown in (a) and (b) respectively. Abbreviations used: IEF, isoelectric-focusing; SDS/PAGE, SDS/polyacrylamide-linear-gradient-slab-gel electrophoresis (see the text for details).

analysis of the immunoprecipitates from cells radioiodinated in the absence or presence of galactose shows the lectin subunits discoidin Ia and Ib as well as discoidin II when the gels are stained with Coomassie Blue (Figs. 3a and 3b respectively). As with the one-dimensional gel analysis, most of the stained polypeptides represents carrier discoidin added before the immunoprecipitation. Autoradiography of the stained gels indicates that radioiodinated discoidin Ia and Ib are present when radiolabelling is carried out in the absence of galactose (Fig. 3c), and hence both these subunits are represented on the cell surface. Radioiodinated cell-surface discoidin II is also resolved by the two-dimensional gel analysis, but is only a faint spot here, in agreement with the preponderance of radiolabel in cell-surface discoidin I over that in discoidin II (Fig. 1b, lane 3). Both discoidins Ia and Ib, as well as discoidin II, are eluted from the cell-surface by galactose as shown by the absence of spots in the autoradiograph (Fig. 3d) of the corresponding gel (Fig. 3b).

Fig. 4. Two-dimensional polyacrylamide-gel electrophoresis of discoidin I radioiodinated *in vitro* bound to *D. discoideum* cells (10h developmental stage) and then eluted from the cell surface with galactose

This figure shows an autoradiograph of the second-dimensional polyacrylamide slab gel after electrophoresis. For details, see the text. For abbreviations, see Fig. 3.

Cell-surface receptors for both discoidin Ia and Ib?

When aggregating *D. discoideum* cells were washed with 0.5 M-galactose/0.075 M-NaCl/0.075 M-sodium/potassium phosphate buffer, pH 7.2, to remove endogenous cell-surface discoidin (Figs. 1b and Fig. 3d) and then incubated with discoidin I radiolabelled *in vitro*, both discoidin Ia and Ib subunits were found to be bound to the cell surface and were specifically eluted with galactose (Fig. 4). Native discoidin I is tetrameric (Simpson *et al.*, 1974). Hence interpretation of these binding data in terms of the presence of receptors for either or both

Fig. 5. Sucrose-gradient sedimentation analysis of discoidin I

Discoidin I, radioiodinated *in vitro*, was analysed by sedimentation (165 000g, 14h, 4°C) in 5–20% (w/v)-sucrose gradients, either (a) in the absence of, or (b) in the presence of, 2% Nonidet P40 and 2% Ampholines. Concanavalin (Con A) tetramer and bovine serum albumin (BSA) were sedimented in parallel gradients to serve as molecular-size markers. Other details are given in the Materials and methods section.

Fig. 6. Two-dimensional polyacrylamide-gel electrophoresis of native and denatured discoidin I (a) Autoradiograph of the second-dimensional gel after analysis of native discoidin I radioiodinated *in vitro* in the presence of 0.3 M-galactose. (b) Autoradiograph of the second-dimensional gel after analysis of denatured discoidin I radioiodinated *in vitro*. For abbreviations, see Fig. 3.

subunits requires a knowledge of the tetramer structure of discoidin I. Conceivably only homologous tetramers Ia₄ and Ib₄ can form, or only hetero-tetramers composed of equal numbers of each subunit to give Ia₂Ib₂, or finally all combinations of the two subunits may occur to give the series Ia₄, Ia₃Ib₁, Ia₂Ib₂, Ia₁Ib₃, Ib₄. If only homologous tetramers form, then the binding data would argue for cell-surface receptors for both Ia and Ib. At the other extreme, heterotetramers of composition Ia₂Ib₂ only would still cause binding of equal amounts of each subunit to the cell surface even if only one type of subunit has an appropriate sugar-binding site for those cell-surface receptors which are present. To clarify this situation, we analysed both native and denatured discoidin I by two-dimensional polyacrylamide-gel electrophoresis. As shown by sucrose-gradient-sedimentation analysis (Fig. 5), native discoidin I molecules remain tetrameric (mol.wt. approx. 110000; Madley & Hames, 1981) in the presence of the Nonidet P40/Ampholine mixture used in the isoelectric-focusing procedure of O'Farrell (1975) provided that urea is omitted; although discoidin I shows a slightly decreased sedimentation velocity in the presence of Nonidet P40, it continues to co-sediment with the concanavalin A tetramer (mol.wt. approx. 112000; Lis & Sharon, 1973) and ahead of bovine serum albumin (mol.wt. approx. 68000).

Two-dimensional gel analysis of discoidin I where the native tetramer was electrofocused in the first dimension in the absence of urea and then dissociated and size-fractionated by electrophoresis in

the presence of urea, SDS and galactose, shows that the native tetramer behaves as a single component with a pI of approx. 6.1 (Fig. 6a). That this indeed represents the native molecule is demonstrated by the lack of a spot in the second-dimensional gel when galactose is omitted from the electrophoresis buffers (I. C. Madley & B. D. Hames, unpublished work). Under these conditions the native discoidin I tetramer elutes from the first-dimensional isoelectric-focusing gel but then binds to the agarose used to seal the first-dimensional gel in place over the second-dimensional gel. If the discoidin I is first denatured and then analysed by essentially the same two-dimensional protocol, but with urea present in the first dimensional gel, the usual two spots characteristic of discoidin Ia and Ib subunits are observed with pI values of approx. 5.9 and 6.3 respectively (Fig. 6b).

Discussion

Two-dimensional gel electrophoresis of native discoidin I yields only a single spot, whereas a similar analysis of denatured discoidin I readily resolves discoidin Ia and Ib subunits and these stain with Coomassie Blue with approximately equal intensity. Our studies indicate pI values of approx. 5.9 and 6.3 for denatured Ia and Ib subunits respectively, somewhat lower than those reported by Tsang *et al.* (1981), and a pI of approx. 6.1 for native discoidin tetramers, in agreement with the value obtained by Simpson *et al.* (1974) and midway between our estimates for the pI of the denatured

subunits. Collectively these data suggest that discoidin I tetramers each comprise approximately equal proportions of Ia and Ib subunits. Since a *D. discoideum* mutant, HJR-1, is known that synthesizes only inactive discoidin I (Ray *et al.*, 1979) yet shows reversion characteristics typical of a single-point mutation (Shinnick & Lerner, 1980), it is possible that a discoidin I tetramer requires both unimpaired Ia and Ib subunits for activity. However, our conclusion that discoidin I exists as heterotetramers remains tentative, since, despite the significant difference in the pI values for denatured discoidin Ia and Ib subunits, it is conceivable that native Ia and Ib subunits possess very similar pI values when in the tetrameric state so that the homologous tetramers Ia₄ and Ib₄ and intermediate forms Ia₃Ib₁ and Ia₁Ib₃, if they exist, could co-focus in the first dimension and so yield a single spot after the second-dimensional separation. An additional complication is that Tsang *et al.* (1981) have been able to resolve discoidin Ib into two single spots on some gels, suggesting that discoidin I may in fact consist of three polypeptides. As shown by the present data, we have not observed this phenomenon. In agreement with Tsang *et al.* (1981), we find only a single spot for denatured discoidin II subunits after two-dimensional gel electrophoresis, so that discoidin II tetramers appear to be constructed of identical subunits, although sequence analysis may show heterogeneity for this lectin also.

The present study clearly shows that both discoidin I and discoidin II are represented on the surface of aggregating *D. discoideum* cells. Furthermore, most of each lectin (at least 92% of discoidin I and 72% of discoidin II) can be eluted from the cell surface with galactose and hence these lectins must be bound to cell-surface receptors via their sugar-binding sites. This finding, which agrees with the data recently provided by Springer *et al.* (1980), supports our assertion made during a previous study (Madley & Hames, 1981) that quantification of the number of cell-surface discoidin receptors can only be achieved with accuracy if the cells are washed with galactose, or another suitable sugar, to remove bound endogenous lectin before receptor assay. Our data also show that the cell-surface form of discoidin I, like intracellular discoidin I, contains both Ia and Ib subunits. Unfortunately, even when galactose-washed cells are used, it is not possible to deduce whether cell-surface receptors exist for only one or both types of discoidin I subunit; assuming that discoidin I is comprised of heterotetramers of Ia and Ib subunits, both Ia and Ib subunits would be found attached to the cell surface even if only one type of subunit had the correct sugar specificity to bind to those cell-surface receptors which are present. Lectins comprised of dissimilar subunits with different receptor-binding activities have already been

characterized in other systems (e.g. Miller *et al.*, 1975). Experiments to determine whether separated renatured Ia and Ib subunits can both bind to the surface of aggregating *D. discoideum* cells will be needed to answer this question. Interestingly, purpurin, the endogenous lectin of *Dictyostelium purpureum*, is also partially cell-surface-located, and more than 90% of this can be eluted with lactose (Springer *et al.*, 1980), a specific sugar competitor for purpurin binding. The attachment *in vivo* of endogenous lectins to cell-surface receptors may therefore be a general phenomenon amongst the cellular slime moulds.

The role of cell-surface discoidin is unknown. Several lines of evidence, most recently the characterization of *D. discoideum* mutant HJR-1, which lacks both active discoidin I and the ability to form tight aggregates (Ray *et al.*, 1979; Shinnick & Lerner, 1980), have been cited in support of a potential role for at least discoidin I in mediating cell cohesion. Since we find that essentially all of the cell-surface-located discoidin I is bound to cell-surface receptors rather than being present as in integral membrane protein, any direct mediation of cell cohesion by this lectin must involve discoidin I tetramers acting as a molecular bridge between the cell-surface receptors of opposing cells. However, evidence for discoidin I as a direct mediator of cell cohesion is still lacking (Springer & Barondes, 1980). Alternatively, discoidin I may be involved in cell cohesion in a regulatory capacity (Marin *et al.*, 1980) as is an endogenous, galactose-specific lectin in *Geodia cydonium* (Müller *et al.*, 1979).

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