Microbes and Infection, 2, 2000, 643–649

S1286457900003610/REV

Review

Cell polarization and adhesion in a motile pathogenic protozoan: role and fate of the *Entamoeba histolytica* Gal/GalNAc lectin

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ABSTRACT – The human pathogenic protozoan *Entamoeba histolytica* is a motile cell polarized into a front pseudopod and a rear uroid. The amoebic Gal/GalNAc surface lectin is a major adhesion molecule composed of an immunodominant 170-kDa heavy subunit, mostly extracellular except for a short cytoplasmic tail, and of an extracellular light subunit. The binding of multivalent ligands triggers lectin capping and recruitment to the uroid. The properties of the Gal/GalNAc lectin and its role in amoeba adhesion and uroid polarization are reviewed in the context of the molecular mechanisms underlying cell polarization and locomotion. © 2000 Éditions scientifiques et médicales Elsevier SAS

Entamoeba histolytica **/ pathogenesis / lectin / cell adhesion / cell movement**

The capacity of living cells to sense their environment is a fundamental feature of biological behavior. Extracellular stimuli activate cellular receptors that trigger an integrated cell response. A directional stimulation, most often chemotactic, regulates polarization of motile eukaryotic cells, eventually promoting an oriented locomotion. It is important to note that stimulation is not required for locomotion and that sensing can occur in the absence of movement, showing that the two processes can occur independently [1]. Coordination of chemotactic sensoring and cell movement depends on intracellular signalling. An integrated view of these mechanisms is being developed for microorganism model systems like the social amoeba *Dictyostelium discoideum* and budding yeast *Saccharomyces cereviseae* [2], and also for the analogous process of cell migration during development, inflammatory processes, metastasis formation (tumorogenesis), or wound healing in higher eukaryotes [3].

Polarized cells present one or several regions that are morphologically and functionally distinct from the remaining part of the cell. These regions are characterized by a differential distribution of cytoplasmic organelles and molecules. Regulation of cell polarization by sensing of a chemoattractant gradient along the cell surface, for example, can provide the stimulus for persistent vectorial motion. This motion seems to be achieved by stabilization of a dominant pseudopodial protrusion to the detriment of other membrane extensions initiating at different regions of the cell [4]. The sequence of molecular events coupling chemotaxis to directional movement is initiated by out-in signalling through activated membrane molecules like the seven-transmembrane-domain G-protein-coupled receptors. Following stimulation, the chemotactic receptors remain evenly distributed in the cell surface (e.g., *D. discoideum*, macrophages) or redistribute to the leading edge of the motile cell (e.g., neutrophils, lymphocytes) [2, 5]. Receptor stimulation is transmitted to the cell interior, leading to conformational changes of their cytoplasmic domain associated with phosphorylation-dephosphorylation events in numerous cases, and/or with interactions with other membrane proteins [1, 2, 5]. This localized signalling is transmitted downstream to regulatory molecules targeting, to a great extent, actomyosin cytoskeleton dynamics (reviewed on [1, 2, 5]). Accumulation of filamentous actin (F-actin) and of a subset of actin-binding proteins at the cell leading edge is an early event during pseudopod formation. Localized actin polymerization, stabilized by bundling and cross-linking proteins, most likely provides the main force driving membrane extension at the cell front [3].

The dynamic interplay between cell adhesion, the substrate where the cell moves, and the cytoskeletal contractile apparatus that is necessary for cell movement has been best characterized for migration of fibroblasts and keratocytes on extracellular matrix. In these systems traction requires the coordinated establishment of adhesive contacts with the substratum at the cell front and their release at the rear during cell body movement. Strong cell-

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substratum attachment points are ensured by adhesion molecules (integrins) in a high-affinity state that bind to the extracellular matrix at the leading edge [3, 6]. Integrins, which cluster upon interaction with substrate, bind a group of specific proteins through their cytoplasmic tails, creating a linkage to the subcortical cytoskeleton which is an organized meshwork of actin filaments (stress fibers). The assembled intracellular complex stabilizes each extracellular adhesion point and results in a strong, yet dynamic, link between the substrate and the cytoskeleton [6]. This structural arrangement seems optimized to convert contractile force generated intracellularly by active myosin motors into traction that drives the cell body forward, resulting in net movement relative to the substrate. Such force can be uncoupled from the pseudopodial membrane extension event [3]. The ratio of contractile force to adhesiveness appears to be the major determinant of cell movement speed which, in turn, tends to be inversely proportional to the contractile force exerted [3]. Detachment of the cell rear from the substrate is thought to result from a combination of a higher contractile force generated by myosin II motors at the cell rear, helping to break down adhesive points by physical stress, and reduction in adhesiveness [6]. Intracellular signalling promotes disassembly of the cytoskeletal links to integrins' cytoplasmic domains in this region of the cell and is also associated with a reduction in the affinity of integrins for their extracellular ligands (inside-out signalling). A significant number of adhesion molecules are, nevertheless, left behind on the substratum during fibroblast movement [3].

The interplay between highly organized cell adhesion points and actomyosin cytoskeletal structures is observed in a number of animal cell systems. This interplay is optimized to support metazoan development and the homeostasis of the multicellular organism. Cell surfaces and extracellular matrix serve as substrates for cellular adhesion molecules whose interactions must be highly specific and regulated for sucessful arrangement of cells in a complex and dynamic architecture. Protein-protein interactions are responsible for most of the highly specific adhesive interactions (e.g., cell-cell contacts: cadherins, other proteins of the immunoglobulin superfamily and integrins on leukocytes; cell-matrix contacts: integrins) while protein-carbohydrate or carbohydrate-carbohydrate contacts appear to mainly facilitate and provide physical strength to adhesion (e.g., cell-cell contacts: selectins in leukocyte traffic; cell-matrix contacts: syndecans) [7, 8].

Unicellular eukaryotes, as compared to metazoan cells, must interact with different and more diverse extracellular substrates. The actomyosin cytoskeleton, however, is universally used to anchor adhesion molecules and to generate contractile force. The adhesion structures and actin filament organization in protozoans like *Entamoeba histolytica* and *D. discoideum* appear less complex than in metazoan cells ([9, 10] and references therein). The properties of protozoan adhesion molecules characterized to date do not allow their classification within the typical classes of animal surface molecules. In spite of these differences, the adhesion mechanisms that protozoans employ also seem highly suitable for interaction with and motion on the substrata they encounter in their natural habitats. Free-living amoeba like *D. discoideum* interact with other microorganisms which are phagocytized as food, with different environmental surfaces (e.g., soil particles), and with other cells of the same species to form multicellular structures [10]. This lifestyle clearly requires adhesive specificities different from those of metazoan cells ([10] and references therein). Another interesting case is that of pathogenic amoeba phylogenetically closely related to free-living amoeba but adapted to live, sometimes exclusively, in the body of their host. Here we review current information on the best characterized adhesion molecule of the intestinal human parasite *E. histolytica* (galactose/*N*-acetyl-D-galactosamine [Gal/GalNAc] inhibitable lectin) in the context of protozoan cell polarization and adhesion mechanisms. The Gal/GalNAc lectin is a heterodimer that binds with high affinity to polyvalent ligands present at the surface of epithelial cells or in mucins. This interaction probably clusters lectin heterodimers and generates out-in signalling through the amoeba membrane. Did the adhesion machinery of this organism require only fine tuning to adapt to its new environment or were significant changes – changes that resulted in the development of adhesion strategies that mimick those of the host cell – required for successful colonization?

1. Cell polarization and adhesion of the pathogenic protozoan *E. histolytica*

E. histolytica is an anaerobic protozoan that causes amoebic dysentery (amoebiasis) in humans, a major disease in developing countries. Primates are the only reservoir epidemiologically relevant for multiplication of the parasite. The parasite is transmitted from one individual to another by parasite-containing cysts resistant to environmental changes [11]. Excystation in the human intestine yields the trophozoite infective form. The trophozoite is a motile amoeba (~ 25 μm diameter [11]) that colonizes the large bowel, feeding essentially on bacteria. Occasional invasion of the intestinal parenchyma at the onset of infection, triggered by still unknown factors, is initiated by adhesion of the amoebae to epithelial cells, followed by contact-dependent cell killing and phagocytosis. Exposed extracellular matrix is extensively degraded by amoebic secreted proteases. Local invasion of the colon results in ulcer formation and eventual dissemination of the pathogen to other organs, which most commonly causes amoebic liver abscess. Chemotactic sensing and the coordinated processes associated with directional locomotion appear central to initiate amoebic infection.

Motile *E. histolytica* trophozoites exhibit a front pseudopod and a rear uroid *(figure 1)*. Amoeba polarization is associated with the differential distribution in the cell of actomyosin complexes and of a group of proteins that regulate cytoskeleton dynamics. The Gal/GalNAc lectin (see below) and some less characterized surface molecules also distribute asymmetrically in the plasma membrane upon activation (a listing of *E. histolytica* surface molecules can be found in [12]). The spatial (re)organization of other molecules that are likely to be involved in *E.*

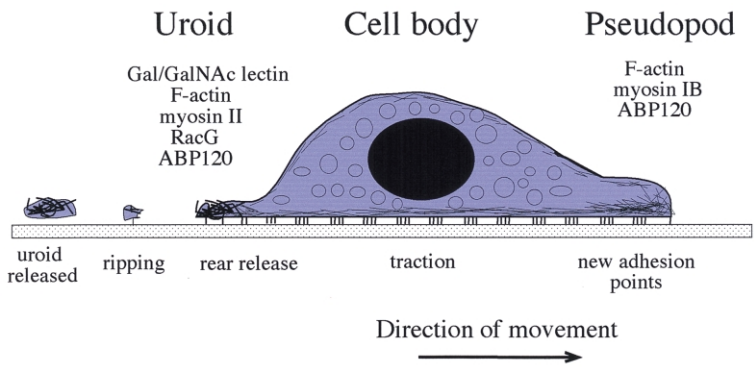

Figure 1. The *E. histolytica* polarized trophozoite. The amoeba is characterized by the presence of a large nucleus (black circle) and abundant vesicles distributed within the cytoplasm (transparent circles). A pseudopod at the front of the moving cell and a uroid at the rear are represented. Molecules presently known to be enriched in one or both of those regions of the cell [9] are listed above the amoeba schema. Subcortical actin filaments, typically concentrated on the pseudopod and in the uroid, are represented by thin lines. Vertical bars connecting the amoeba to the substrate represent adhesion points. The different events associated with cell motion [6], including the proposed release of membranous material at the cell rear (please refer to main text), are indicated in the lower part of the figure.

histolytica polarization, by analogy to other cell systems, has not yet been characterized. These include surface receptors and adhesion molecules other than those reported above, microtubules, and the cellular components dedicated to membrane trafficking pathways necessary for endocytosis of surface molecules [4].

The *E. histolytica* pseudopod is characterized by a local accumulation of F-actin, of the F-actin cross-linker ABP120, and of unconventional myosin IB [9]. Twofold overproduction of myosin IB interferes with cytoskeleton dynamics at the pseudopod front, inhibiting erythrophagocytosis, but does not affect cell locomotion [13]. Chemotaxis or phagocytosis receptors expected to direct vectorial extension of the amoebic pseudopod have not been identified. No evident substrate attachment points were observed at the cell front during pseudopod formation (our unpublished observations) and the adhesion molecules that cooperate with the cytoskeleton components are, as yet, unknown. Following pseudopod extension, the protrusion either retracts and pseudopods emerge at other positions in the amoeba, or cytoplasmic streaming and cell body movement occur towards the pseudopod region, resulting in net cell motion. As found in other cell types, conventional myosin II plays a central role in *E. histolytica* trophozoite movement [14].

The amoeba uroid is a structure at the cell rear characterized morphologically by large amounts of folded membrane [15]. This structure is detectable in a small number of the trophozoite population grown under axenic conditions. Binding of multivalent ligands (e.g., antibody-containing serum from amoebiasis patients or the tetravalent lectin concanavalin A) to surface molecules leads to the formation of uroids in the majority of an amoebae population [16]. First, the binding of multivalent ligands causes clustering of surface molecules that are presumably cross-linked by the ligand in an energy independent process as described for lymphocytes and *D. discoideum* [17, 18]. These clusters are actively transported in a rearward direction to the posterior part of the moving trophozoite where they coalesce to form large patches (caps). Movement of receptor-ligand clusters is prevented by inhibition of myosin II mechanochemical activity [14]. Actin, myosin II, actin-binding proteins (e.g., ABP-120 and proteins related by immunological criterium to spectrin, α-actinin, and ezrin ([9]; N.G. and P. Arhets, unpublished observations) and the putative actin cytoskeleton regulator RacG, a homolog of the human small GTPase Rac1 [19], concentrate below the caps *(figure 1)*. The role of the actomyosin cytoskeleton in capping is further confirmed by expression of a dominant negative form of myosin II that blocks uroid formation in transfected amœba [14]. Caps accumulate in the uroid structure, which is then released from the amoeba [16]. Contractile forces generated at the cell rear by the locally enriched myosin II motor might facilitate uroid release. The end result of capping is thus the elimination of multivalent ligands bound at the parasite surface. This is likely a strategy for the trophozoite to subvert host immune effectors.

The *E. histolytica* Gal/GalNAc inhibitable lectin is an immunodominant surface glycoprotein that mediates adhesion to human colonic mucins and to epithelial cell monolayers. In polarized amoeba, it exhibits a somewhat patchy distribution that appears to be reduced in the extending pseudopod (unpublished observations), while capping promotes its recruitment to the uroid [9]. The lectin amino acid sequence, substrate-binding properties, and the few known cellular events triggered during adhesion share some common features with other surface molecules but do not allow its classification within any of the known classes of such molecules described for higher eukaryotes. The Gal/GalNAc lectin is also implicated in evasion of the host immunoresponse during invasive amoebiasis. The molecule mediates resistance to human complement C5b-9 ([20] and references therein) and clearance of polyvalent antibodies bound to the parasite surface by capping and uroid release.

2. Structural organization of the Gal/GalNAc lectin

The Gal/GalNAc inhibitable lectin is a glycoprotein composed of one light (Lgl; 31 or 35 kDa) and one heavy subunit (Hgl; 170 kDa) stabilized by disulfide bonds [21]. The purified nonreduced lectin has an apparent mass of 260 kDa in SDS-PAGE. Two Lgl forms with different electrophoretic mobilities assemble into distinct heterodimers with Hgl [22]. A family of genes located at six distinct loci within the amoeba genome [23] code for the Lgl isoforms which share significant identity in amino acid sequence and composition but exhibit distinct posttranslational modifications. While the 31-kDa subunit has a carboxyl terminus acyl-GPI anchor that presumably attaches it to the plasma membrane, the 35-kDa subunit does not appear to contain the glycolipid but undergoes more extensive glycosylation [22]. No antibody able to bind to Lgl is detected in serum of patients during invasive amoebiasis or recovering from amoebiasis, probably because the light subunit remains protected by the immunodominant large extracellular region of Hgl [21].

Five potential different genes (*hgl1* to *hgl5*) coding for Hgl were identified (89–95% amino acid identity [23–25]). Hgl exhibits some immunological and weak amino acid sequence similarity to extracellular and cytoplasmic domains of higher eukaryotes' surface molecules ([24, 25, 26] and references therein; N.G., unpublished results). Sequence analysis revealed the organization shown in *figure 2*. The lectin heavy chain extracellular portion (LHCEP) region is characterized by regions of high cysteine content and by posttranslational modifications, the most extensive being N-linked glycosylation [25]. LHCEP glycosylation and preponderance of cysteine residues, providing the potential to form disulphide bonds, ensure stability of the folded lectin at the amoeba surface exposed to the harsh environment of the gut and to the amoebic secreted proteases [25]. The data compiled in *figure 2* clearly define an immunodominant region within the Hgl cysteine-rich area that is likely to represent the surface exposed to the extracellular environment. This region includes a carbohydrate-binding site with binding specificity similar to the one found for the complete lectin and for amoeba membranes [27]. A stretch of hydrophobic residues (putative transmembrane domain) preceeds the 39 carboxyl-terminal amino acids that presumably form the lectin heavy chain cytoplasmic domain (LHCCD) *(figure 2)*. Mutagenesis of four amino acids localized in the central region of the LHCCD *(figure 2)* demonstrated their importance for lectin-mediated adhesion to epithelial cells [26] (see below). Neither ligands nor interaction partners of the LHCCD have been reported yet. The described properties of the lectin heavy chain and studies using animal models ([28] and references therein) have made Hgl a major candidate for a vaccine against amoebiasis.

3. Ligand-binding properties of the Gal/GalNAc lectin

The strong inhibition of *E. histolytica* adhesion to epithelial cells by millimolar amounts of Gal or GalNAc was the first evidence for the central role of a lectin in cell-cell interactions. Trophozoites adhere poorly to target cells defective in production of N- and O-linked Gal-terminal oligosacharides, confirming this carbohydrate specificity [29]. The 260-kDa Gal/GalNAc inhibitable lectin binds to mucus glycoproteins and accounts for most of the adhesive capacity of the microbe to several types of mammalian cells [26, 27, 30, 31].

The Gal/GalNAc lectin binds polyvalent N-acetylgalactosaminides with an affinity in the nanomolar range. Affinity is increased dramatically when molecules with a polyvalent nonreducing terminal of either Gal or GalNAc are used as ligands. Galactose inhibitable binding of purified mucins to amoeba, for example, has a dissociation constant of 8.2×10^{-11} M^{-1} [31]. Using competitive inhibition assays of ligand-binding to *E. histolytica* isolated membranes, Adler et al. [30] and Yi et al. [32] defined the interaction properties, namely the ionic requirements for micromolar amounts of calcium or for hundred millimolar concentrations of NaCl. The best binding was observed with ligands in which a repetition of GalNAc residues was spaced within a core molecule (e.g., $GalNAc_n$BSA neoglycoproteins, where 'n' represents an average number of GalNAc residues conjugated per bovine serum albumin molecule). The optimal spatial geometry between GalNAc residues was not defined, but best results were obtained when the spacing was quite wide, suggesting that the lectin binds to sacharide 'maxiclusters' that might mimic the structure of intestinal mucins [32]. Thus, high-affinity binding seems to require the interaction of multiple lectin molecules in the amoebic membrane with a multivalent carbohydrate target. This interpretation is in agreement with the large number of ligand-binding sites (2.8×10^3) found per amoeba [31]. The hepatic lectin of mammalian host cells also exhibits Gal/GalNAc binding specificity. However, some specific structural requirements in ligand structure and the spacing between nonreducing terminal sacharides in the multivalent ligand are different when the Gal/GalNAc lectin and the mammalian hepatic lectin are compared [32]. This distinction is of therapeutic relevance because the *E. histolytica* lectin can specifically be targeted without interfering with host cell adhesion events.

4. The Gal/GalNAc lectin in amoebic adhesion

The Gal/GalNAc lectin mediates the dynamic interaction of amoeba with epithelial cells. Monoclonal antibodies (mAbs) binding to the cysteine-rich LHCEP inhibit or enhance amoeba adhesion [33]. The epitopes of inhibitory and stimulatory mAbs overlap partially [33] *(figure 2)*. Binding of mAbs might act sterically, masking the lectin carboydrate-binding site (inhibition), or might promote lectin conformations that reduce (inhibition) or facilitate binding (stimulation). These data suggest that the lectin is conformationally flexible and that it can switch between conformations with different ligand-binding properties, as proposed for integrins [34]. mAb binding would stabilize a specific conformation or directly promote acquisition of

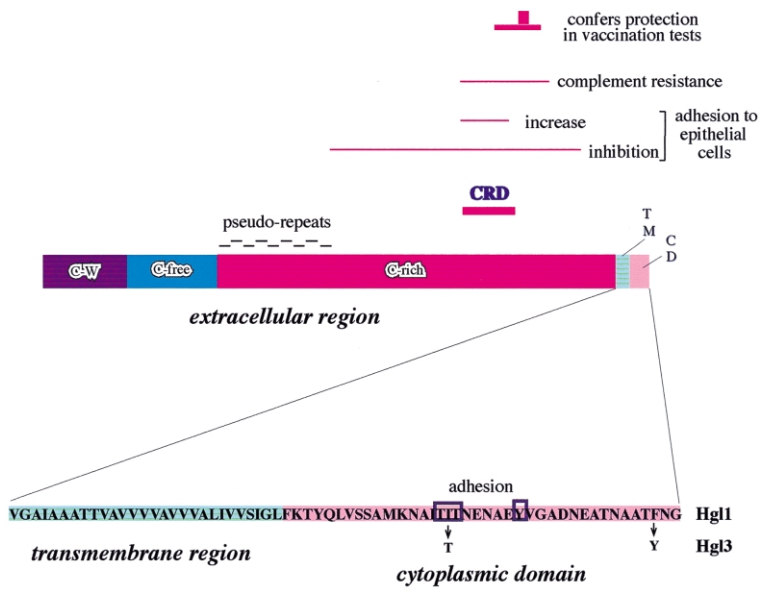

Figure 2. The heavy chain (Hgl) of the Gal/GalNAc inhibitable lectin from *E. histolytica*. The protein has a molecular mass of 143 kDa based on the amino acid composition derived from the gene nucleotide sequence and an apparent mass of 170 kDa based on its electrophoretic mobility. The central bar represents the structural organization of Hgl based on its primary structure [24, 25]. The cysteine-tryptophan-rich (C-W rich: 3.2% cysteine and 2.1% tryptophan of the total amino acid composition), cysteine-free (C-free), cysteine-rich (C-rich: 10.8% cysteine), transmembrane region (TM) and the lectin heavy chain cytoplasmic domain (CD) are represented to scale. Nine contiguous pseudorepeats of ~ 30 amino acids are identified by lines above the cysteine-rich region [24]. The regions involved in adhesion to epithelial cells and resistance to complement [33] are identified by thin lines on the upper part of the schema, while the carbohydrate recognition domain (CRD) [27] is indicated by a thick bar. The very probable exposure of this area at the Hgl surface was further confirmed by the identification of a region (thick upper bar) that confers protective immunity against amoebic liver abcess in gerbils [28]. The red square shows the most immunogenic peptide of the protective polypeptide sequence. The sequence of the Hgl putative transmembrane region and cytoplasmic domain is shown in the figure's lower part. Amino acid differences between the isoforms Hgl3 [24] and Hgl1 [25] are indicated. Four residues implicated in amoeba adhesion to epithelial cells [26] are boxed.

such a three-dimensional fold. Switching between ligand high- and low-affinity states of adhesion, normally modulated by intracellular signals that are transmitted across the membrane to the extracellular region, allows for coordinated cycles of binding and release from the substrate during cell locomotion (*figure 1*; see above). Considering the high affinity of the lectin for its polyvalent ligands, such a mechanism is likely necessary to allow for recycling of the lectin which probably occurs by endocytosis at the cell rear. A significant amount of lectin is nevertheless lost from the amoeba surface. Lectin originating from trophozoites cocultured with epithelial cell monolayers was found associated to the basolateral membrane of epithelial cells localized underneath the amoeba [35]. Furthermore, a Hgl chain slightly smaller than the membrane-associated form is found in culture supernatants [36] and is probably the result of shedding due to the amoeba's own proteases. Trophozoites were also observed to release membrane vesicles from the rear pole enriched in capped surface molecules (uroids). These losses imply that continuous biosynthesis of lectin is necessary to maintain its pool at the membrane surface.

The adhesive properties of the Gal/GalNAc inhibitable lectin play a role during different steps of *E. histolytica* colonization of the gut and further invasion. The trophozoite is initially trapped in the intestinal mucus, a viscous gel-like layer composed of a complex mixture of components including glycoproteins (mucins) with nonreduced terminal Gal and GalNAc that bind with high affinity to the lectin. Amoebic movement is slowed down and mucin-binding inhibits lectin interaction with enterocytes. Trophozoites stimulate mucus production, leading eventually to exhaustion of mucus secretion or alteration of the protective mucosal layer. Amoeba then gain access to the apical surface of the underlying enterocytes [31]. The interaction of trophozoites with enterocytes has been mimicked in vitro using epithelial cell monolayers. Adhesion is followed by contact-dependent cytolysis of epithelial cells. The Gal/GalNAc inhibitable lectin is necessary for adhesion, which can occur at 4 °C, and also plays a role in the cytotoxic effect observed uniquely at physiological temperature [20]. Reduced production of the lectin light chain in genetically engineered trophozoites inhibits specifically their cytotoxic activity without affecting adhesion to epithelial cells [37]. This phenotype correlates to a significant decrease in virulence, providing the first evidence for a role of Lgl in invasive amoebiasis [37, 38]. It remains to be determined whether the lectin acts to ensure a persistent close physical contact between the trophozoite and target cell membranes necessary for delivery of the cytotoxic effectors or if it participates directly in disruption of the host cell membrane. Interaction of amoebas with

epithelial cell monolayers leads to transfer of the Gal/GalNAc inhibitable lectin, or at least a part of the molecule, to the basolateral region of the epithelial cells at the level of the adhesion junctions [35]. This interaction requires live trophozoites and epithelial cells, suggesting an active mechanism that either destabilizes the monolayer and permits direct access of the lectin to the basolateral region of the epithelial cell or that leads to the transport of epithelial cell surface molecule-lectin complexes to that region [35].

5. Perspectives in Gal/GalNAc lectin research

The binding properties exhibited by amoeba membranes indicate that multiple lectin heterodimers are cross-linked by the polyvalent ligand. This local clustering most probably generates out-in signalling causing the observed actin accumulation in the cytoplasmic cortical region underneath the interaction area [20]. Vines et al. [26] showed that intracellular expression of the lectin cytoplasmic domain specifically inhibits trophozoite adhesion and identified some of the LHCCD essential residues involved *(figure 2)*. These residues are conceivably necessary for interaction with cytoplasmic titrable factors that regulate lectin-mediated adhesion, and three of them are potential targets for phosphorylation events. Even though phosphorylation of the LHCCD was not detected, a related 170-kDa lectin from the free-living amoeba *Hartmannella vermiformis* has been demonstrated to undergo tyrosine phosphorylation-dephosphorylation events [39]. The molecular mechanisms controlling (i) how these features converge to promote an integrated cellular response, (ii) which regulatory and cytoskeletal molecules are recruited to support coordinated adhesion and amoeba motion, and (iii) how the lectin adhesive properties are modulated by intracellular signalling are questions that will be undoubtedly tackled in the near future.

Acknowledgments

We thank Michelle Rathman and Elizabeth Labruyère-Dadaglio for critical reading of the manuscript. P.T. is supported by an EMBO Long Term Fellowship. Work of our laboratory on the *Entamoeba histolytica* project is supported by grants from the French Ministère de l'Education Nationale, de l'Enseignement Supérieur, de la Recherche et de l'Insertion Professionelle (MNER), and from the NORD-SUD INSERM program (grant 4N0016).

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