Oktoberfest for adhesion structures

Meeting on Adhesion

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The Adhesion Meeting 2005 was organized by S. Linder, R. Fässler, E. Genot and P. Jurdic, and took place in Munich between 28 and 30 April 2005. The booklet of abstracts and a picture gallery of the meeting can be accessed at: www.adhesion-meeting.com

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year. Almost 20 years after the term 'podosome' was coined (reviewed in Linder & Aepfelbacher, 2003; Buccione *et al*, 2004), a lively crowd of international researchers from the different areas of cell adhesion convened in Munich to discuss the similarities and differences of podosomes, invadopodia and focal adhesions (Fig 1).

Focal adhesions mediate the stable attachment of cells to the underlying substratum and anchor the actin cytoskeleton at the inner face of adherent cells. Podosomes are most prominent in cells derived from the monocytic lineage, such as macrophages, osteoclasts and dendritic cells, but they have also been described recently in epithelial, endothelial, and even smooth muscle cells. These adhesion structures seem to be required for physiological processes such as the migration of phagocytes, smooth muscle cells and dendritic cells, and the adhesion, migration and invasion of endothelial cells during the formation of blood vessels. Podosomes have also been proposed to be the precursors of invasive and matrix-degrading organelles such as the osteoclast ring and the invadopodia of tumour cells. These latter structures form protrusions at the ventral surface of invasive tumour cells, and they coincide with degradation sites of the underlying ECM. Despite their different structural and functional features, focal adhesions, podosomes and invadopodia share many of the same molecular components (Fig 2; Table 1).

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Introduction

Cell motility and tissue invasion depend on the interaction of the cell with its surrounding environment, such as with neighbouring cells, the extracellular matrix (ECM) and even inorganic bone components (for a review, see Geiger *et al*, 2001). Focal adhesions have long served as a model system to study cell–matrix interactions, but other adhesion structures such as podosomes and invadopodia are made by different cell types and are heterogeneous in their molecular composition, structure and function. This apparent complexity of adhesive structures sparked the first Adhesion Meeting earlier this

Podosomes versus invadopodia

Cells embedded in a soft environment develop adhesion structures that are largely different from those found on stiff substrates in cell culture. Therefore, it has been questioned whether the short-lived and dynamic podosomes studied *in vitro* actually resemble a physiological structure. By contrast, invadopodia are more persistent and are thus more likely to mediate cell movement across tissue boundaries.

R. Buccione (Santa Maria Imbaro, Italy) suggested that podosomes on a stiff, non-degradable support are “frustrated invadopodia” that are unable to penetrate an inflexible matrix. Furthermore, Buccione reported that invadopodia of metastatic cancer cells are unable to form when intracellular transport is blocked by brefeldin A, and also ECM degradation is prevented when matrix metalloproteinases (MMPs) are blocked. This suggests that polarized secretory transport is a prerequisite for MMP-dependent focalized matrix degradation.

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J. Evans (Cambridge, MA, USA) added another dimension to the investigation of podosomes by using four-dimensional (4D) deconvolution microscopy. Using the actin filament cross-linking protein fimbrin fused to the green fluorescent protein (GFP) as a reporter, he analysed the actin turnover of macrophage podosomes using kymography (a 3D graphical representation of time-related events). He found that podosomes assemble and turn over by a process of continued fission from, and fusion back to, larger podosome precursors. Using automated, high-content imaging based on measurements of cell shape and actin distribution, Evans is now developing an informatics-based approach to create morphological signatures of podosomes for the high-throughput analysis of potential therapeutic compounds.

Drebrins are a family of actin-binding and -remodelling proteins that are especially enriched at the junctional plaques of polar epithelial cells. The podosomes of primary human umbilical vein endothelial cells (HUVECs), studied by S. Linder (Munich, Germany) and his team, appear in dynamic wave-like structures that contain drebrin. The inhibition of drebrin expression by RNA interference (RNAi) strongly inhibits podosome formation, which highlights the potential importance of this class of actin regulatory molecules for podosome assembly.

Osteoclasts are specialized cells that resorb mineralized bone during bone remodelling. Their filamentous actin pool is organized in two ways: as a peripheral belt of podosomes and as a 'sealing zone', which is a large band of actin with an inner and outer lining of vinculin that forms close to the perinuclear region. The sealing zone delineates the area of enzyme secretion and matrix degradation in osteoclasts, and the analogies between podosome belts and the sealing zone in both shape and molecular composition have led to the hypothesis that the sealing zone is derived from the podosome belt by fusion. Osteoclast podosomes were thought to be essential for ECM degradation and bone resorption by providing sites of focal lytic activity. However, MMP-mediated ECM degradation at podosomes and bone degradation at the specialized sealing zone in the centre of the cell might represent different processes. A detailed analysis by M. Chellaiiah (Baltimore, MD, USA) showed that the phosphatidylinositol 4,5-bisphosphate (PtdInsP₂)-generating enzyme, phosphoinositide 3OH-kinase (PI3K), associates with the actin-filament-severing protein gelsolin. Furthermore, osteopontin (OPN), a glyco-phosphoprotein that regulates cell-matrix interactions through binding to integrin receptors, stimulates PI3K activity. Gelsolin deficiency blocks podosome assembly and motility in osteoclasts, but the cells still exhibit matrix resorption. These findings support the idea that podosomes are necessary for the migration of osteoclasts, but might be dispensable for bone resorption.

The doubts about the bone-matrix-resorbing function of podosomes were substantiated further during the meeting, and P. Jurdic (Lyon, France) essentially shattered our current beliefs about the function of these structures. By observing osteoclasts on a mineralized apatite matrix (Saltel *et al.*, 2004), Jurdic's group has shown that the sealing zone does not mature from podosomes and might be an entirely new structure. The actin ring rapidly disintegrates during the spreading of osteoclasts, which seem to undergo a resorption-migration cycle. The sealing zone forms on the transition towards a stationary phase that is coupled with active matrix degradation. Jurdic showed that during osteoclast migration through stromal cells, actin patches, but not podosomes, are formed. Thus, there seems to be a functional and temporal difference between invasive osteoclasts and resorbing osteoclasts.

Fig 1 | Different types of cell-extracellular matrix contact structures.

(A) Elongated focal adhesions (FAs) in a stationary adhesive cell. (B) Peripheral focal complexes (FXs) and anchoring FAs in a protruding cell. (C) Podosomes (yellow) in smooth muscle cells. (D) Podosome rosettes (yellow) in epithelial cells. (E) Podosomes can aggregate to rings. (F) Aggregates of podosome rings can form belt-like structures. (G) Invadopodia (red; arrowheads) in a cell grown on a fluorescent extracellular matrix substrate (green) form preferentially in the centre of the cell. Images were provided by M. Gimona (A, B, C, E, F), S. Linder (D), and R. Buccione and I. A. Grande (G).

The regulation of actin polymerization is considered to be a key pathway that leads to cell migration. Activation of the Arp2/3 complex by members of the Wiskott-Aldrich syndrome protein (WASp) family mediates actin filament assembly. H. Yamaguchi (New York, NY, USA) has developed a fluorescence resonance energy transfer (FRET) biosensor that tracks the ubiquitous N-WASP variant. He used this method to analyse invadopodium formation, and noted some differences in the assembly of invadopodia and podosomes. He suggested that the short-lived, motile invadopodia are equivalent to podosomes, which are

Fig 2 | Differences between adhesion structures. Focal adhesions (1) anchor bundled actin filaments at the sites of integrin clustering and extracellular matrix (ECM) contact. By contrast, podosomes (2) and invadopodia (3) are characterized by high actin polymerization activity and short, disorganized actin cores surrounded by a ring of adhesion structures. Whereas the podosome cores are dynamic and have a minute-scale turnover rate, invadopodia can remain stable for several hours and invade the ECM as a consequence of excessive matrix degradation. Green spheres, pieces of ECM removed from the substrate by MMP activity; grey circles, vinculin-rich adhesion sites; white areas, regions of complete ECM degradation.

precursors of fully functional invadopodia, and presented a three-step model for invadopodium formation: initiation; searching and anchorage; and, finally, maturation and matrix degradation.

Mechanosensation: adhesions on a flexible leash

Mechanical probing of the immediate environment of the cell is a crucial mechanism that controls several processes including motility, morphogenesis and proliferation. Therefore, another theme of the meeting was the molecular and functional characterization of the mechanosensing machine (Geiger & Bershadsky, 2001, 2002) that converts signals and information about the contractile state of the cell into a molecular and cellular response. Indeed, a sophisticated mechanosensory device detects forces and enables cells to respond to them, and there is compelling evidence that integrin-

mediated adhesions are central to the mechanosensory process. Mechanical forces exerted by the contractile actomyosin system of a cell (Galbraith & Sheetz, 1998) guide the maturation and size of focal adhesions. The correlation between force and focal-adhesion size suggests that they have a mechanosensory function. One of the target molecules for the mechanosensor is the histone deacetylase HDAC6, and A. Bershadsky (Rehovot, Israel) proposed that this molecule forms a universal link between the two main cytoskeletal systems—actin filaments and microtubules. According to Bershadsky's model, the diaphanous-related formin Dia1 has an important mechanosensory role, and the interaction between Dia1 and HDAC6 could create flexible links between growing microtubule ends and actin filaments (that is, form a 'flexible leash'). This would reduce microtubule dynamics, facilitate microtubule targeting to focal adhesions and provide a negative feedback loop necessary for the control of focal-adhesion size.

Focal-adhesion composition is complex because of the number of proteins that localize to the adhesion structures, and due to the numerous, varied interactions that these molecules can make. Suggesting that temporal events in cell matrix adhesions are responsible for the complexity of focal-adhesion composition, B. Geiger (Rehovot, Israel) proposed that certain molecular switches (such as phosphorylation or proteolysis) dictate adhesion assembly. As an example, he described the cellular response to shear stress, during which a hierarchical assembly of focal complexes takes place. Geiger also discussed the formation of 'primordial' or 'soft' adhesions, which form in the absence of detectable focal-adhesion components and which might be driven by integrin-mediated ECM interactions (Zaidel-Bar *et al*, 2003). A quantum dot approach showed that the matrix molecule that is probably responsible for these interactions is hyaluronic acid. In the hierarchical scheme of adhesion progression, the initial formation of soft contacts by hyaluronic acid and the subsequent collapse of the hyaluronic-acid gel prepares the stage for the formation of focal complexes and early contacts, which then mature into stabilized focal adhesions.

Another hierarchical principle was highlighted in the discussion of the role of integrins in mechanotransduction. Traction forces (Katsumi *et al*, 2004) provide a crucial mechanism by which the ECM promotes migration of Madine–Darby canine kidney (MDCK) cells out of epithelial islands. M.A. Schwartz (Charlottesville, VA, USA), together with collaborators C. Waterman-Storer and J. de Rooij, discovered that the activation of $\alpha\beta3$ -integrin in stretched fibroblasts on elastic substrates is mediated by PI3K, and that enhanced binding to the ECM and PI3K activity seem to be both necessary and sufficient for integrin activation. This study provides the first mechanistic explanation for the regulation of cell scattering by mechanical forces.

Cell adhesions serve as traction sites for migration as the cell moves forwards. The assembly and disassembly of adhesions requires the coordinated interaction of the actin cytoskeleton with signalling molecules and several enzymes including proteases, kinases and phosphatases. Integrin-linked kinase (ILK) is an adaptor protein that links the cytoplasmic domains of integrins with cytoskeletal components and is thought to be involved in the reorganization of the actin cytoskeleton. E. Boulter (Nice, France) showed that membrane-targeted ILK activates the small Rho GTPases Rac and Cdc42. Eliminating ILK expression by RNAi resulted in defects in cell spreading, membrane protrusion and adhesions to fibronectin. An ILK mutant (targeted to membranes

Table 1 | Some of the molecular components of focal adhesions, podosomes and invadopodia

Component	Function
Integrins (e.g. $\alpha v\beta 3$, $\alpha 2\beta 5$, $\alpha 3\beta 1$, $\alpha 6\beta 4$)	Dimeric transmembrane receptors linking ECM and cytoskeletal elements
Actin	Cytoskeleton filament component
Fimbrin	Actin filament cross-linking protein
Drebrin	Actin-binding protein
Gelsolin	Actin filament-severing protein
Formins (e.g. Dia1, Dia2)	Multidomain proteins nucleating polymerization and mediating the formation of linear actin filaments
Arp 2/3 complex	Protein complex initiating polymerization of branched actin filament
Cortactin WASp/N-WASp	Activator of Arp2/3 activity
Talin Paxillin Vinculin	Focal adhesion component

ECM, extracellular matrix; WASp, Wiskott–Aldrich syndrome protein.

through the addition of a GFP-farnesyl anchor) increased cell spreading and induced lamellipodia and Rac activation. Farnesyl-ILK-GFP also drove paxillin into elongated focal adhesions to increase the cell surface, whereas expression of the non-farnesylated ILK reporter did not. Thus, ILK seems to have a central integrating role for the formation or stabilization of the mechanosensor by mediating the regulation of cell-matrix adhesion dynamics.

Nanolimbo: how low can you go?

Measuring the contractile forces exerted by a single cell and the manipulation of cell–matrix adhesions requires sophisticated tools for the preparation of patterned, non-homogeneous substrate surfaces to which the cells adhere. Using silicon wafers to produce patterned surfaces of defined size, B. Hinz (Lausanne, Switzerland) showed that substrate elasticity levels are inversely related to focal-adhesion size. A surface size of $20\ \mu\text{m} \times 1.5\ \mu\text{m}$ induced the generation of supermature focal adhesions (with sizes up to $30\ \mu\text{m}$) and incorporation of α -smooth muscle actin (α -SMA) into the stress fibres. Reducing the size of the patterned surface to below $4\ \mu\text{m} \times 1.5\ \mu\text{m}$ restricted focal-adhesion growth to normal (mature) size ($2\ \mu\text{m}$ – $6\ \mu\text{m}$), and α -SMA was not incorporated. Hinz proposed that matrix tension enlarges focal adhesions and increases stress fibre size, and that incorporation of α -SMA into stress fibres requires a stress perception threshold of $>50\ \text{nN}$ per focal adhesion.

J. Spatz (Heidelberg, Germany) has developed a nanoscale method in which 8-nm gold particles are coated with RGD tripeptides and are organized at different distances from each other. The size of these particles allows them to interact with only one integrin receptor, and when they are placed at a distance of 58 nm apart, fibroblasts adhere, develop paxillin- and zyxin-rich focal adhesions and an organized actin cytoskeleton, and migrate. By contrast, when the particles are at a lateral distance of 110 nm, the cells only weakly adhere, fail to stimulate adhesions to mature into larger sized focal adhesions and have a disorganized actin cytoskeleton. A. Cavalcanti-Adam (J. Spatz's group) explained how these cellular behaviours are caused by the restricted clustering of

integrins, and she estimated the maximal lateral limiting distance for integrin clustering to be 58 nm–73 nm.

Combining the micropattern approach with high-resolution total internal reflection fluorescence (TIRF) microscopy, U. Joos (Berlin, Germany) investigated the influence of structured surfaces on cell guidance. Using electron-beam lithography, she generated surface patterns that resemble the natural distances between individual focal adhesions in cells. Cells that adhere to the surface adopt the pattern within 12 hours, preferentially orientate their actin cytoskeleton in parallel to the adhesion pads, and even migrate along the tracks determined by the pattern.

Knockouts

Integrins are fundamental to cell–cell and cell–ECM interactions, and integrate both structural and signalling elements required for adhesion formation and dynamics. Targeted deletion of individual integrins and the components with which they interact can reveal unexpected insights into integrin function. While visiting the laboratory of R. Fässler (Munich, Germany), D. Bouvard (La Tronche, France) generated a constitutive knockout of the integrin cytoplasmic domain-associated molecule1 (ICAP1). ICAP1 interacts specifically with the cytoplasmic domain of $\beta 1$ -integrin, and acts as a messenger between sites of cell adhesion and the nucleus for controlling gene expression and cell proliferation. ICAP1 deletion affected $\beta 1$ -integrin function in osteoblasts and elicited severe defects in proliferation and mineralization. This suggests that ICAP1 is involved in the regulation of osteoblast function and that ICAP1-dependent $\beta 1$ -integrin signals have a key role in osteogenesis.

M. Kretzler (Munich, Germany) generated a podocyte-specific conditional ILK-knockout mouse. These mice develop progressive proteinuria and glomerulosclerosis, and die at approximately 16 weeks. Ultrastructural analysis showed a striking alteration in glomerular basement membrane assembly followed by obliteration of podocyte foot processes. This approach promises important insights into the function of ILK, as the gradual onset of this phenotype will allow ILK to be evaluated under physiological conditions.

The disease dimension

Mutations in the *WAS* gene strongly impair normal WASp function and result in diseases with complex phenotypes. However, the main features of Wiskott–Aldrich syndrome seem to result from aberrant cell migration. WASp is a central component of leukocyte podosome cores, and macrophages and dendritic cells from patients suffering from the disease fail to produce $\beta 2$ -integrin-rich podosomes. These cells therefore migrate abnormally through the use of focal adhesions (G. Jones, London, UK). Ectopic expression of GFP–WASp restores podosome formation in WAS cells and reintroduces directional cell motility. Migration is also important for dendritic cell function, and normally these cells move through the production of a lamellipodium. In *WASp*^{−/−} cells, however, lamellipodium formation is defective, and transmigration is severely impaired *in vitro* (S. Burns, London, UK). As these cells also lack podosomes, $\beta 2$ -integrins are abnormally localized at the cell cortex/periphery, which leads to compromised adhesion to intercellular adhesion molecule 1 (ICAM1) *in vivo*. Notably, cells from WAS patients and the WASp-knockout mice have similar defects in migration. Burns thus concluded that podosomes are important for normal dendritic-cell trafficking and transmigration through the endothelial barrier, and that they stabilize the protrusions that form.

Infectious pathogenic microbes require the host-cell cytoskeleton for their initial uptake and further dissemination, and modulate the integrin-related adhesion machinery for the formation of entry sites. Whereas several bacteria, such as *Yersinia enterocolitica*, are able to bind directly to integrin receptors, *Staphylococcus aureus* expresses fibronectin-binding proteins (FNBP-A and -B), which mediate the engagement of integrin receptors and the recruitment of focal-adhesion proteins. The enzymatic and scaffolding function of focal adhesion kinase (FAK) is necessary for the uptake of *S. aureus*, as FAK-null cells show severely diminished integrin-mediated internalization of the pathogen (C. Hauck, Würzburg, Germany). During this process, an active FAK–Src complex modulates the tyrosine phosphorylation of cortactin—an actin-binding protein that is enriched at pathogen attachment sites. Cortactin mutants that are impaired in their ability to bind to the Arp2/3 complex interfere with the uptake process, which supports earlier evidence that the recruitment of the actin polymerization machinery to sites of pathogen adhesion promotes the engulfment of the bacteria into host cells.

Conclusions and perspectives

This meeting had several highlights, particularly when considering the technical advances presented. Even though no breakthrough discoveries were revealed, a few burning questions were raised. For example, when is a podosome an invadopodium (or should one say ‘invadosome’)? The migration of cells through the extracellular matrix exists in both physiological and pathological situations. Invadopodia might, however, resemble a more physiological version of podosomes, and what separates these similar structures is not necessarily their molecular composition but rather the functional differences brought about by maturation and stability. Even osteoclasts seem to switch functionally between migratory and resorptive activities. They might require their podosomes for migration and to prepare cells for firm attachment before the formation of a sealing zone and bone degradation.

Clearly, future discussions will revolve around what one might envisage as a ‘podosome maturation model’. Is there a hierarchical relationship between focal adhesions, podosomes and invadopodia? These divergent structures certainly share many components. Adhesions are highly complex interfaces that produce nodes of multidomain interactions and eventually could form a functional unit. A possible extension of this view could be the definition of an ‘adhesion module’ that can translate similar inputs through processing, using apparently the same molecular/computing components, into different cellular outputs—as exemplified by focal complexes, focal adhesions, podosomes, invadopodia, or dorsal ruffle waves.

This meeting celebrated new enthusiasm and momentum in the field of cell adhesion that will continue to hammer away at old and new boundaries and produce important scientific controversies. And there is more of this to come in the near future as R. Buccione and M. Gimona will host the second Adhesion Meeting in 2007 in Santa Maria Imbaro, Italy.

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