# The Adhesion-Mediated Environment-Sensing Machinery of Living Cells


Department of
Molecular Cell Biology

**Prof. Benjamin Geiger**

Michal Brunner, Dafna Geblinger, Carlos Hung, Masha Khoutorsky, Irina Lavelin, Mati Mann, Suha Naffar Abu-Amara, Yael Paran, Ziv Porat, Ilana Sabanay, Tova Volberg, Sabina Winograd-Katz, Haguy Wolfenson

☎ 972 8 934 3910
FAX 972 8 946 5607
@ benny.geiger@weizmann.ac.il
www.weizmann.ac.il


## Why adhesion?

Adhesion to the extracellular matrix (ECM) or to neighboring cells regulates multiple cellular processes such as cell migration, morphogenesis, proliferation, gene expression and survival. Activation of these responses involves specific interaction of membrane bound adhesion receptors with the external surface, sensing its chemical, topographic and mechanical features, activating of specific signaling networks and inducing the assembly of multi-protein adhesion complexes at the contact sites. Research activity in the group addresses a wide variety of topics associated with the structure and function of cell adhesions, and the mechanisms whereby they serve as environmental "sensorsy devices".

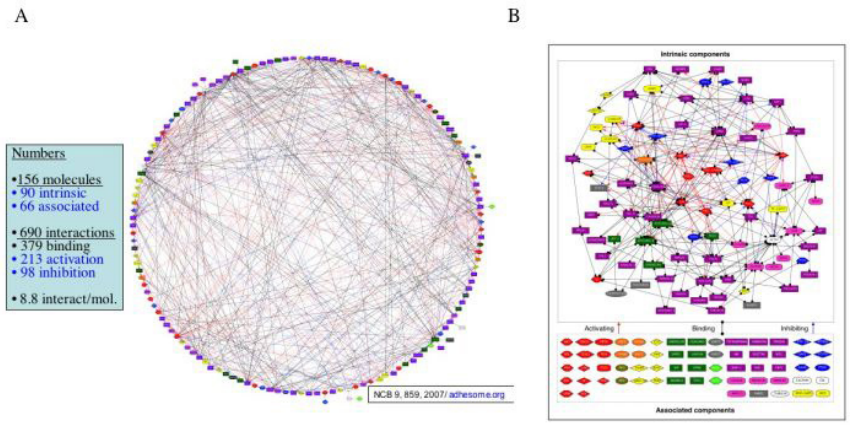


***Fig. 1*** *A. Complexity of the adhesome, depicting the binding and modification interactions between the molecular constituents of the adhesome. B. The right panel shows the different "families" of adhesome components, distinguishing between intrinsic and associated molecules (Zaidel-Bar et al., 2007).*

## Functional molecular anatomy of the "adhesome" network

Integrin-mediated adhesions are highly complex structures, consisting of multiple extracellular and cellular components, associated with the actin cytoskeleton. To date more than 150 molecules were reported to be associated with integrin adhesions, and participate in their assembly and signaling activities. The "adhesome" network was found to be highly interconnected, offering docking sites for signaling molecules, and apparently regulating a multitude of adhesion-dependent processes [Figure 1 and see (Zaidel-Bar et al., 2007) and http://adhesome.org]. What are the particular roles of individual components in the complex adhesome network? To address this question, members of the group develop and use genetic tools to over express or eliminate specific target molecules, and check the consequent effects on cell behavior and fate. These include knocking-down the expression of individual genes, using siRNA transfection approach, and determining the effects on adhesion site development and dynamics. Another current study is exploring the src-induced mechanisms, responsible for cytoskeletal reorganization and modulation of adhesion sites.

## The sensory functions of adhesion sites

Cells can sense a wide variety of environmental (chemical or physical) cues via their adhesion sites. Several lines of experiments currently running in the lab, are aimed at the elucidation of diverse surface features that can differentially affect the adhesion sites. These include systematic screening for variations in surface chemistry that can be sensed and distinguished by cells, the effects of precise inter-ligand spacing, matrix rigidity, roughness and more. Using advanced quantitative microscopy, and a variety of functional assays we are determining the effects of these surface features on cell spreading, polarization, focal adhesion shape and dynamics, activation of specific signaling networks and more (Spatz and Geiger, 2007). Studies addressing the design and synthesis of "smart" adhesive matrices are conducted in collaboration with Joachim Spatz (MPI, Stuttgart, Germany). Another feature of cellular mechanosensitivity is related to the effect of mechanical forces on adhesion sites. Studies in collaboration with A. Bershadsky demonstrated that application of force to focal adhesions induces their growth and/or apparent "migration". These observations stimulated the development of several theoretical models, based on different physical considerations, and detailed studies of the particular signaling pathways that are regulated by mechanical forces, and their capacity to alter the molecular interactions within the adhesome, or within the associated signaling systems.

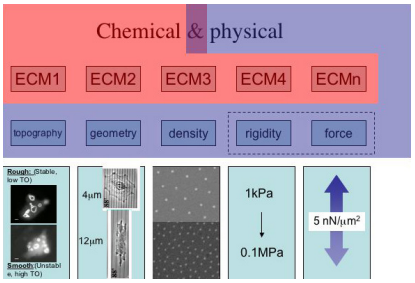


***Fig. 2*** *A scheme, showing the chemical and physical aspects of adhesion-mediated signaling, including the responses to different adhesive ligangs (ECM 1-n) and the differential cellular response to surface roughness, geometry, ligand spacing, substrate rigidity and applied forces.*

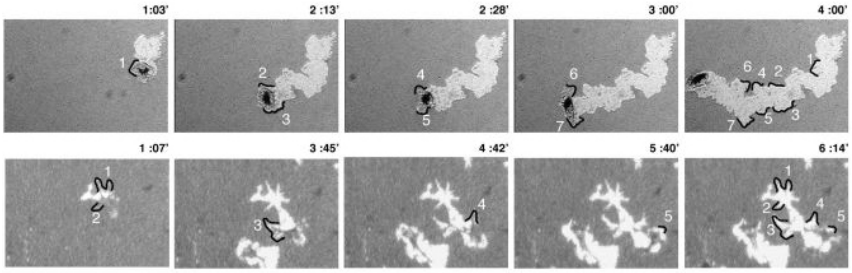


***Fig. 3*** *The formation of phagokinetic tracks, by two cell types, with different migratory features. The figure shows several frames from a time-lapse movie (times are marked), marking specific protrusive events.*

**Regulating cell adhesion and migration in cancer**

Comprehensive screens for genes involved in the regulation of cell migration are carried, using high-throughput microscopy (see below). In these screens, stationary cells were plated on a monolayer of microbeads, after being transfected with either genes derived from a highly metastatic breast carcinoma cell line (MDA231 MB) or a cDNA collection, encoding a variety of breast cancer related proteins (BC1000). Cell migratory activity was monitored and quantified by analyzing the "phagokinetic tracks" formed by the cells [Figure 3]. Candidate genes are now being evaluated for their role in cancer invasion and metastasis (Naffar-Abu-Amara et al., 2008). Variations in cell adhesion and migration are also investigated in multiple myeloma (in collaboration with B. Katz, Tel Aviv-Sourasky Medical Center). In these studies it was discovered that transformed plasma cells (line ARH-77) form heterogeneous cultures, contain an adhesive (type A) and non-adhesive (type F) sub-populations. Type A and F cells were shown to differ in their migratory activity, surface markers, gene expression profiles and tumorigenic activity in mice. Interestingly, the differences between these lines appear to be non-genetic, and each sub-population can re-diversify into both types following cultivation under non-selective conditions (Nadav et al., 2006). The mechanisms underlying the transformation from one form to the other, as well as the relationships between the adhesive phenotype and the tumorigenic properties of these cells are currently under investigation.

**Mechanisms of bone remodeling**

A particular form of integrin-mediated adhesions, which is prominent in monocyte-derived and src-transformed cells are the podosomes. Osteoclasts, whose primary function is bone resorption, organize podosomes in a particular structure known as the "sealing zone". In our studies, carried out in collaboration with Lia Addadi, we address a variety of topics related to the effects of the chemical and physical properties of the adhesive surfaces on stuctural and dynamic features of podosomes, in osteoclasts [see figure 4]. These include cell polarization and spreading, assembly of a sealing zone like structure, differential interaction with bone and artificial surfaces, podosome dynamics and more (Luxenburg et al., 2006). The involvement of the cell signaling machinery in podosomal reorganization and dynamics is further investigated in cells over-expressing a deregulated src-kinase. By silencing specific genes in these cells, we are identifying specific src-targets and src-dependent pathways that are involved in podosome and sealing zone assembly. In an additional line of experiments, we are testing, in collaboration with Eran Hornstein, the role of micro-RNA in podosome differentiation and function.

**Adhesion-dependent regulation of cell life and death**

Cell adhesion to the ECM plays a central role in supporting cell survival. Thus – most non-transformed cells, when deprived from an appropriate matrix adhesion, and cultured in suspension, undergo programmed cell death. What are the adhesion-mediated signals, which support cell survival, and how is cell death induced in their

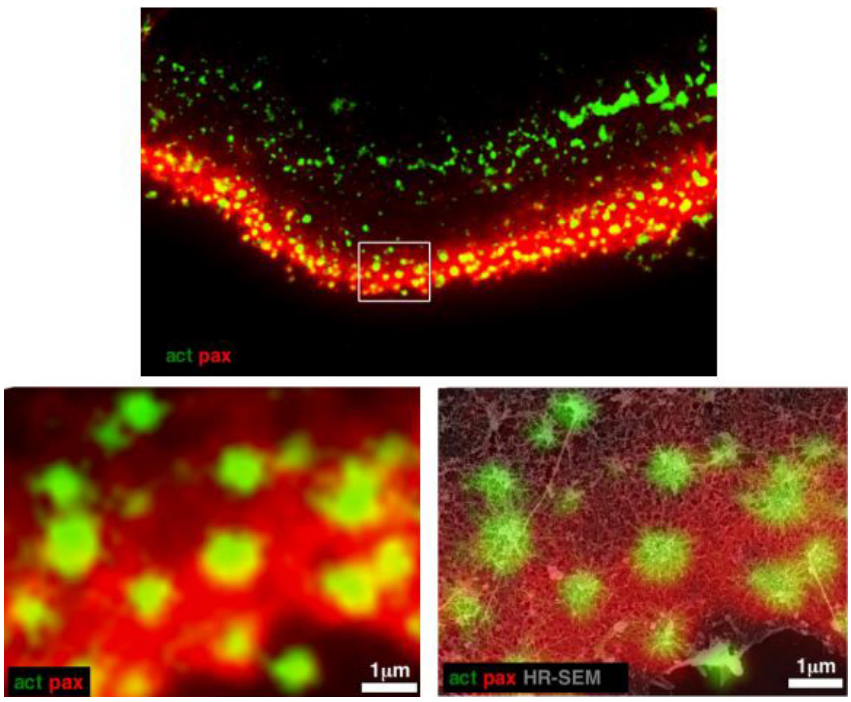


***Fig. 4*** *Podosomes, cultured of osteoclasts, visualized by expression of fluorescent actin (green) and paxillin (red). Lower panels show an overlap between the fluorescence image, and high-resolution scanning electron microscopy of the corresponding ventral membrane (Luxenburg et al., 2007).*

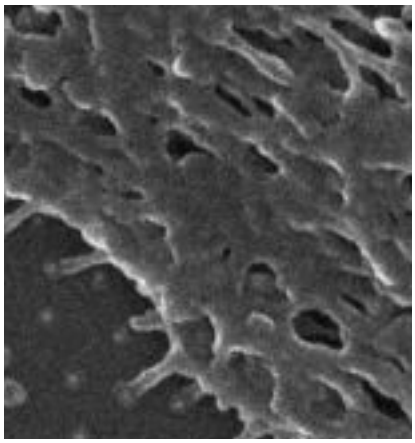

***Fig. 5*** *Association of cultured cells with nanostructured surfaces (from the lab of J. Spatz) (Cavalcanti-Adam et al., 2007; Spatz and Geiger, 2007).*

absence? A related question is how do transformed cells become "anchorage independent", and can grow without being associated with the matrix? Studies, currently in progress in the lab, use siRNA knockdown approach in an attempt to interfere with genes that induce death in normal cells, which are forced to grow in suspension, or genes that allow survival of anchorage-independent cells. A siRNA approach is also used, in collaboration with Yehiel Zick for investigating genes involved in the programmed death of pancreatic beta cells, exposed to death-inducing cytokines.

## Matrix formation and modulation

The formation of the ECM is a complex process, in which attached cells are actively participating. In the case of fibronectin (FN), for example, cells secrete this matrix molecule, then bind to it and pulls it, forming large fibrils. In this process of pulling these elastic fibers – high affinity binding sites are exposed on the FN molecules, further enhancing the adhesive interaction. To explore the role of mechanical force on FN fiber formation experiments were conducted, in collaboration with the laboratory of Joachim Spatz in Stuttgart, where droplets of FN solution were placed on top of an array of hydrophobic micropillars, and subjected to mechanical stress. Examination of the pillars by light and electron microscopy revealed highly ordered arrays of FN fibers, which interconnect the pillar tops. The dimensions and shape of the fibers depend on the FN concentration, and the level of force applied to the droplet. These experiments were followed by real time monitoring of FN fiber formation in cells, expressing a fluorescent derivative of FN. To explore the genetic basis for FN fibrillogenesis, we are currently checking the effect of systematic knock down of many gene families on the assembly of FN fibers.

## Correlated microscopy

A major challenge in modern biology is the development of a bridge between light microscopy (which enables live cell imaging, and has a diffraction-limited resolution of down to ~0.2μm) and high-resolution electron microscopy (which required fixation, and approaches molecular resolution). To span this large gap in resolution we grow cells on glass slide or an EM grid, examine them first in the light microscope, and then process the specimen for electron microscopy. This approach was first applied by us to cultured osteoclasts, revealing the organization of the podosomal unit [(in collaboration with Ohad Medalia) (Luxenburg et al., 2007), Figure 4]. Recently, we extended this approach to focal adhesions, revealing the fine organization of these adhesion sites, visualizing the attached cytoskeleton as well as specialized multi-protein complexes, located at the interface between the plasma membrane and the cytoskeleton.

## Nanobiological approach for engineering of cellular environments

A set of powerful approaches recently employed in our laboratory is based on the application of nanoscopic approached for studying cell adhesion. For example, it was shown by the Spatz lab that nano-patterned surface with a spacing of 58 nm can support cell adhesion and spreading, while a somewhat larger spacing (>70nm) lacks this capacity [Figure 5 and (Cavalcanti-Adam et al., 2007; Spatz and Geiger, 2007)]. This approach is currently used by us, addressing the behavior of cells on a variety of adhesive nanopatterns, including gradients of ligand density, elastic nanopatterned surfaces and gold nanopatterns, functionalized with a variety of adhesive peptides. Cells types examined for the behavior on these surfaces include fibroblasts, different epithelia, endothelial cells, osteoclasts, muscle progenitors, a variety of cancer cell lines and mesenchymal stem cells.

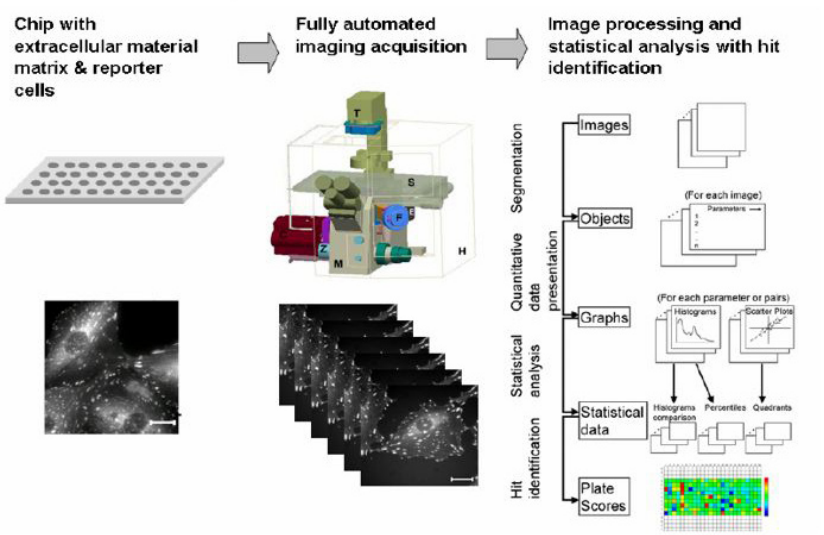


***Fig. 6*** *General framework of high throughput screens, utilizing the high-resolution screening microscope. It demonstrates (left to right) the screening multiwell plates or "cell chips", the reporter cells, the automated microscope, with its different components and the image processing structure (from data acquisition to plate scoring).*

**Quantitatibve automated microscopy and high throughput screens**

In collaboration with Z. Kam an advanced system for automated quantitative microscopy was developed and applied for several screening projects, including discovery of novel adhesion proteins, search for modulators of proteasome action, search for drugs inducing cancer cell death, genes affecting cell migration, siRNA modulating cellular features as focal adhesions, FN fibrillogenesis, podosome formation by src-transformed cells, drug effects on microtubules and more [see Figure 6].

**Selected publications**


Cavalcanti-Adam, E.A., Volberg, T., Micoulet, A., Kessler, H., Geiger, B. and Spatz, J.P. (2007) Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands. Biophys J, 92, 2964-2974.

Luxenburg, C., Geblinger, D., Klein, E., Anderson, K., Hanein, D., Geiger, B. and Addadi, L. (2007) The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. PLoS ONE, 2, e179.

Luxenburg, C., Parsons, J.T., Addadi, L. and Geiger, B. (2006) Involvement of the Src-cortactin pathway in podosome formation and turnover during polarization of cultured osteoclasts. J Cell Sci, 119, 4878-4888.

Nadav, L., Katz, B.Z., Baron, S., Cohen, N., Naparstek, E. and Geiger, B. (2006) The generation and regulation of functional diversity of malignant plasma cells. Cancer Res, 66, 8608-8616.

Naffar-Abu-Amara, S., Shay, T., Galun, M., Cohen, N., Isakoff, S.J., Kam, Z. and Geiger, B. (2008) Identification of novel pro-migratory, cancer-associated genes using quantitative, microscopy-based screening. PLoS ONE, 3, e1457.

Spatz, J.P. and Geiger, B. (2007) Molecular engineering of cellular environments: cell adhesion to nano-digital surfaces. Methods Cell Biol, 83, 89-111.

Zaidel-Bar, R., Itzkovitz, S., Ma'ayan, A., Iyengar, R. and Geiger, B. (2007) Functional atlas of the integrin adhesome. Nat Cell Biol, 9, 858-867.



***Acknowledgements***

Erwin Netter Professor for Cell and Tumor Biology; Research support from NIH, EC, ISF, BSF, GIF, DIP, Minerva; Stem Cell Center, VW Foundation, The Helen and Martin Kimmel Institute for Stem Cell Research, The Women's Health Research Center, The Jeanne and Joseph Nissim Foundation for Life Science Research, The J&R Center for Scientific Research