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The Molecular Basis for Cell Adhesion

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The molecular complexity of Focal Adhesions

Adhesive cellular interactions regulate the assembly of cells into tissues and organs, control cell motility, affect cell shape and orchestrate the activation of diverse signaling networks. FA are specialized integrin-mediated, actin-bound cell- matrix contacts. In a series of studies by *Eli Zamir*, the molecular diversity of matrix adhesions was characterized, based on quantitative molecular mapping of their different components (Fig. 1). These differences are attributable to the composition and rigidity of the matrix, contractility of the actin cytoskeleton and the combined action of different protein kinases. Molecular interactions in FA are mapped by *Christoph Ballestrem*, using fluorescence resonance energy transfer assay. The functional properties of the different components forming the protein network in FA, is studied by *Miriam Tsalyuk.* Using an RNAi approach, she eliminates specific components of the plaque from cells and determines the effect of this perturbation on the entire network.

FA contain well over 50 known components. *Irena lavelin* is currently looking for novel FA molecules, using a microscope-based genome-wide high-throughput screening. Her approach is based on the infection of target cells with GFP-tagged cDNA library, followed by microscopic selection of clones displaying fluorescent FA, using the automated microscopy system developed by Prof. *Zvi Kam*. One of the genes identified encodes a 72KDa Rac/cdc42-specific GAP protein that affects actin organization. An additional application of the high throughput microscopy system, carried out by *Yael Paran*, is the screening of compound libraries for novel cytoskeleton- and adhesion-modulating drugs.

The first adhesions

Early adhesive interactions between cells and external surfaces are mediated by a pericellular coat consisting of hyaluronan. The molecular properties of this coat and its cooperation with the integrin system are investigated by *Miriam Cohen* (in collaboration with Prof. *Lia Addadi*). Using advanced light- and electron microscopy, Miriam showed that hyaluronan forms a 1-5 micrometers thick, gel-like coat around cells. This coat mediates “soft adhesion” and tethers the cell to the surface prior to the engagement of integrins. The earliest integrin-mediated adhesions during cell spreading are investigated by *Baruch Zimerman* who characterized the transition of early, dot-like adhesions into mature FA. Time-lapse recording of the organization of actin, and different FA proteins, tagged with GFP, revealed a highly dynamic process of FA growth and reorganization. *Ronen Zaidel-Bar* is studying the formation and consolidation of matrix adhesions during cell migration. Monitoring migrating endothelial cells, he

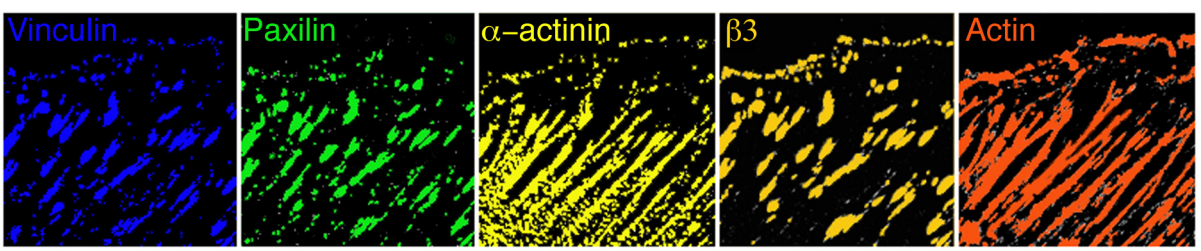

Fig. 1 *Multiple colors labeling of REF52 cells for components of focal adhesions – namely vinculin, paxillin, α-actinin, β3 integrin and actin (prepared by Eli Zamir).*

demonstrated that the assembly of primordial FA (i.e. "focal complexes") and their "maturation" involves a hierarchical recruitment of different plaque proteins, some of which bind to nascent adhesions only when mechanical stress is applied to them.

The extracellular matrix (ECM)

The composition, topography and mechanical properties of the ECM regulate FA assembly. *Baruch Zimerman* is investigating cell adhesion to micro- and nano-patterned surfaces, flat and three-dimensional, elastic and rigid. Monitoring the spreading of cells on such surfaces, he demonstrated the capacity of cells to form new adhesions only when reaching a "permissible" region. Reorganization of the ECM by cells can also affect the assembly of matrix adhesions. *Tova Volberg* is currently studying the effect of anastellin, a modulator of fibronectin fibrillogenesis, on the molecular properties of adhesion sites. She showed that addition of anastellin to cultured fibroblasts induces disassembly of fibronectin fibrils and development of aberrant adhesions.

A special case, where adhesion is directly involved in the primary cell function, is the adhesion of osteocalsts to bone surface. *Chen Luxenburg* characterizes the adhesion of these cells to artificial and physiological surfaces (in collaboration with Prof. *Lia Addadi*). He examines the formation of adhesive rings, made of small "units" called podosomes, showing that the assembly of the resorptive apparatus depends on matrix composition, src-kinase signaling, and cellular contractility.

Adhesion-mediated signaling

Cell adhesions play a dual physiological role: they mediate the mechanical interaction and the generation of signals that regulate cell behavior. *Noam Erez* showed that N-cadherin-mediated adhesion provides critical survival signals, which can be inhibited by cadherin-inhibitory peptide and restored by fibroblast growth factor. He further found that cadherin signaling stimulates cell spreading by src-dependent activation of the small G-protein Rac. Src-mediated signaling is critical for diverse adhesive phenomena. *Tova Volberg* showed that the FAK/src pathway plays an important role in FA turnover. *Chen Luxenburg* discovered that tyrosine phosphorylation regulates podosome dynamics in osteoclasts, and expression of pp60src mutants alters podosome integrity.

Cell migration

Coordinated formation of adhesions and protrusions affects cell migration and tumor metastases. *Suha Naffar Abu-Amara* developed high throughput screens for cell migration, for the discovery of migration-inducing, cancer-related genes. She is quantifying "phagokinetic tracks" following transfection with cDNA from highly metastatic breast cancer cells. Another cancer system where the fine balance between adhesion and migration might be associated with tumor progression is multiple myeloma. *Liat Nadav* (in collaboration with Dr. *Ben-Zion Katz,* TASMC) is studying the migratory mechanism of these cells on various matrices.

Cell adhesion and mechanical Force

Recent studies, in collaboration with Prof. *Alexander Bershadsky*, have shown that FA are mechanosensitive. In addition, *Ronen Zaidel-Bar* is testing the effect of shear flow on the formation and turnover of FA. He showed that the arrest of cell migration against flow depends on local inactivation of Rac, and that FA with different orientation are differentially affected by the flow.

Cell contractility and glaucoma therapy

In a long-term collaboration with Prof. *Paul Kaufman* (U. Wisconsin, Madison) we have explored the possibility of reducing intraocular pressure (which is a major cause of glaucoma) using cytoskeleton-perturbing drugs. The joint studies include physiological measurements and cellular studies. *Ilana Sabanay* showed, using EM, that addition of such drugs to the anterior chamber of the eye causes major reorganization of the flow pathway, suggesting that actin-modulating compounds might be developed into "outflow enhancing drugs" for glaucoma therapy.

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