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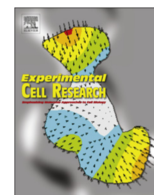

The journey of integrins and partners in a complex interactions landscape studied by super-resolution microscopy and single protein tracking

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Cells adjust their adhesive and cytoskeletal organizations according to changes in the biochemical and physical nature of their surroundings. In return, by adhering and generating forces on the extracellular matrix (ECM) cells organize their microenvironment. Integrin-dependent focal adhesions (FAs) are the converging zones integrating biochemical and biomechanical signals arising from the ECM and the actin cytoskeleton. Thus, integrin-mediated adhesion and mechanotransduction, the conversion of mechanical forces into biochemical signals, are involved in critical cellular functions such as migration, proliferation and differentiation, and their deregulation contributes to pathologies including cancer. A challenging problem is to decipher how stochastic protein movements and interactions lead to formation of dynamic architecture such as integrin-dependent adhesive structures. In this review, we will describe recent advances made possible by super-resolution microscopies and single molecule tracking approaches that provided new understanding on the organization and the dynamics of integrins and intracellular regulators at the nanoscale in living cells.

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1. Introduction

Biomechanical cues are genuine signals regulating cell behavior during cell differentiation, cell motility, ECM remodeling in normal [1,2] and pathophysiological conditions such as cancer, which is often associated with tissue stiffening [3]. In order to sense the physical and biochemical properties of the surrounding extracellular matrix (ECM), cells need to establish specialized adhesive structures with their microenvironment. Cell-matrix adhesions are the mechanical interfaces where mechano-transduction events convert mechanical cues into biochemical signaling. It exists a diverse catalog of adhesive structures to the ECM (namely 'nascent

Abbreviations: ECM, extracellular matrix; FAs, focal adhesions; FCS, fluorescence correlation spectroscopy; FRAP, fluorescent recovery after photobleaching; FRET, fluorescence resonance energy transfer; iPALM, interferometric photoactivated localization microscopy; PALM, photoactivated localization microscopy; SMLM, single molecule localization-based microscopies; SPT, single protein tracking; sptPALM, single particle tracking PALM; STORM, stochastic optical reconstruction microscopy; uPAINT, universal point-accumulation-for-imaging-in-nanoscale-topography

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adhesions', focal adhesions, fibrillar adhesions, podosomes, invadosomes) that differ by their shape, sub-cellular location, lifetime, functions and cell-type specificity [4]. But all these sub-cellular structures rely on the integrin family of adhesion receptors.

Integrins mediate cell adhesion by binding to their extracellular ligands, but also support force transmission to/from the cell by connecting to the actin cytoskeleton. Thus, integrins are obvious players in force and rigidity sensing mechanisms. Consistent with this idea, cells spread on stiff substrates functionalized with integrin ligands adopt a larger final area and develop mature focal adhesions (FAs) compared to soft substrates [5,6]. FAs are dynamic mechano-sensitive platforms that respond to mechanical forces generated either by internal actomyosin contractility or by external ECM stretching. Independently of their origin, increased force leads to FA assembly and decreased force to FA disassembly [7–10].

Integrin receptors, core components of FAs, are heterodimeric transmembrane proteins formed by non-covalently bound α and β subunits. Each integrin subunit has a large extracellular domain that constitutes the ligand binding domain, a single transmembrane domain and a short cytoplasmic tail. Since the short cytoplasmic tails of integrins lack enzymatic and actin-binding activity, they rely on binding to structural and adaptor proteins to establish the physical linkage with the actin cytoskeleton and recruitment of signaling proteins. Numerous proteins compete for few binding sites on the short cytoplasmic tails of β integrins, implying that regulatory processes control the spatiotemporal reversible binding of specific adaptors [11].

1.1. *Adhesome revealed the complex landscape of biochemical and biomechanical interactions network*

Experimental and theoretical inventories of proteins involved in integrin adhesion sites formation and signaling have been termed integrin 'adhesome' and were determined by quantitative proteomic [12–15] or data mining [16]. Such studies built functional and protein–protein interactions maps with components of integrin-based adhesive structures regrouped in functional families including adhesion receptors, adaptor proteins, actin regulators, kinases/phosphatases, Rho GTPases, lipids and proteolytic activities [16]. Now quantitative proteomic approaches explore the average composition of the adhesome depending on specific integrins with differential functions in mechanotransduction (αv-, β1- or β3-classes) [13,17] but also the dependence on myosin-II activity [12,14]. Importantly, the recruitment of proteins into the integrin adhesome could be positively but also negatively regulated by myosin-II-dependent contractile machinery. Such maps revealed that the composition of cell-matrix adhesions is far from unique but display a diverse panel of protein combinations depending on the cellular context. Although such interaction maps can lead to the identification of new regulators of integrin-based adhesion sites, they cannot predict the sub-cellular location, duration, strength, sequence, and nature (competitive versus cooperative) of protein interactions. These parameters are particularly important since macromolecular complexes are built and stabilized from stochastic encounters in the cytosol or at the plasma membrane between nanometer-sized proteins under thermal forces that could funnel into specific interactions.

1.2. *From single particle to single protein tracking (SPT)*

To understand how these molecular interactions are regulated spatially, temporally and mechanically in the cellular context, it is essential to assess the architecture and dynamics of FAs components with spatial resolution at the scale of the proteins themselves. The sequence of protein recruitment leading to FA initiation and maturation is not random. A large body of biophysical studies using tracking of gold nanoparticles, micron-sized latex or magnetic beads attached to integrins on the dorsal part of the cells decrypted what are the biochemical and mechanical determinants inducing connection of integrin-based molecular complexes to actin cytoskeleton. It provided evidence that (1) integrins upon ligand binding switch from free diffusion to rearward directed motion after coupling to the actin retrograde flow [18] supporting integrin "outside-in" signaling; (2) oligomerization of integrins favors such coupling which depends on talin, one of the major integrin activator with actin-binding properties [19,20]; (3) upon restraining mechanical forces, mimicking larger rigidities, this linkage to actin cytoskeleton is reinforced [21] and this reinforcement is dependent on specific integrins and talin [9,22]; and (4) controlled by phosphotyrosine signaling [23–25]. Even though those experimental approaches were crucial in defining the key events leading to adhesive structure mechanotransduction, most of these findings were reporting responses of an ensemble of integrins as beads could potentially bind to several integrins at once. Moreover, they were unable to probe integrins connection to actin cytoskeleton directly within adhesive structure on the ventral part of the cell. Less invasive techniques based on protein labeling in live cell such as fluorescence time-lapses [26], fluorescent recovery after photobleaching (FRAP) [27,28], fluorescence correlation spectroscopy (FCS) [29–31] and fluorescent speckles microscopy [32] revealed the global and internal dynamics of FAs components. FRAP and FCS are based on ensemble measurements in which diversity of molecular behavior remains hindered. Speckle microscopy requires that the studied protein assemble in an immobile or slowly mobile molecular complex, preventing access to the fast dynamics of proteins. In addition, the resolution of fluorescence microscopy (around 200 nm) is fundamentally limited by the diffraction of light. During the last decade, super-resolution fluorescence microscopy techniques revolutionized biomolecular imaging in cells by delivering optical images with spatial resolutions below the diffraction limit of light [33–36]. Among the panel of super-resolution techniques, single molecule localization-based microscopy (SMLM) is achieved by acquiring collections of images containing distinct, sparsely located fluorescent entities while keeping the rest of the population in non-emissive states. This is performed by sequentially activating and localizing (super-resolution) or tracking (SPT) a small number of fluorophores. By repeating this process for thousands of images, the sample can be reconstructed at the nanoscale (PALM, iPALM, STORM) [33,37,38] or trajectory maps of proteins can be built directly in their crowded, confined and pristine environment (stpPALM, uPAINT) [36,39]. Such imaging modalities start now to reveal how the interactions landscape is regulated in time and space inside sub-cellular regions including cell-matrix adhesion structures [40–43].

2. Architecture of cell-extracellular matrix adhesion sites at the nanoscale

Precise spacing between each FA components is a critical parameter in the FA formation and stability. Indeed, the nanoscale spacing and density of integrin ligands were shown to regulate cell adhesion, shape and motility [44–48]. This suggests the existence of precise internal nanoscale architecture inside functional adhesive structures. Evidence for the need of a nanoscale description came with dual-color super-resolution imaging of proteins tagged by genetically expressed fluorescent probes within individual FAs [40]. In this study, the authors revealed that adhesome proteins, which appear co-localized using conventional light microscopy,

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