

## Use of adhesion molecule-coated surfaces to decipher cell contact formation and associated intracellular responses

[R.M. Mège](#)

<sup>1</sup> INSERM U706, Institut du Fer à Moulin, Paris, France.

How cells sense their environment during embryonic tissues formation and adjust their response in terms of shape, proliferation, differentiation and survival remains one of the more puzzling questions in biology, with high physiopathological implication for tissues homeostasis. In the seventies, cytoskeleton linked cell surface glycoproteins (cadherins and integrins) have been discovered that mediate specific cell-cell and cell-matrix adhesion and transduce mechano-chemical signal from cells in contact.

Cadherins are of special interest for the mechano-transduction at cell-cell contact as they act as both their own ligand and receptor and establish a direct link between adjacent cells. Widely expressed and dynamically regulated during development, they control the aggregation and segregation of embryonic cells, the formation of intercellular junctions and cell differentiation. In complex with their intracellular partners (catenins and signaling proteins), they transduce signals leading to cytoskeleton remodeling and more global intracellular signaling.

However, one of the major limitations for cadherin studies, hampering the elucidation of their mode of action, has been the absence of a method to activate these receptors in a controlled manner. In this context, we developed an approach based on the use of immobilized recombinant cad-Fc ligands<sup>1</sup> to activate cadherins precisely, enabling both the study of the dynamics and molecular regulation of cell contact formation<sup>2,3</sup> and demonstrating the usefulness of biomimetic surfaces to progress in the characterization of the cellular response<sup>4,5</sup>.

Using such biomimetic system (Ncad-Fc coated-beads) to specifically activate N-cadherin, we previously described in myogenic C2 cells a fast adhesion-triggered and Rac1-dependent anchoring of N-cadherin to the actin cytoskeleton enabling the transduction of forces generated by the actin tread milling. Recent observations by videomicroscopy in neuronal cells expressing Ncad-GFP (N-cadherin tagged to the Green Fluorescent Protein) indicate that N-cadherin anchoring to the cytoskeleton is followed by a further recruitment of N-cadherin molecules.

We showed previously that cell spreading on Ncad-Fc-coated surfaces induces a strong co-recruitment of cadherin, catenins and actin filaments into focal adhesion-like structures (cadherin adhesions), that we analysed here by RICM (Reflection Interference Contrast Microscopy) and TIRFM (Total Internal Reflection Fluorescence Microscopy). Cadherin adhesions were easily detected by both methods confirming their close apposition to the substratum and their relative stability over time. We followed their formation in real time using GFP-tagged molecules. Tiny clusters of cadherin appear at the tip of growing lamellipodia and then fuse to form cadherin adhesion at the rear of lamellipodia. In some cells, nascent structures were reset and reoriented in relation with global lamellipodial dynamics. Experiments performed with various N-cadherin mutants deleted of their cytoplasm tail confirm that N-cadherin recruitment in cadherin adhesion requires their association to catenins and actin. FRAP (Fluorescence Recovery After Photobleaching) experiments further show that a population of N-cadherin molecules in cadherin adhesion was diffusive while another was submitted to a slow turnover indicative of a regime exchange limited by the formation/dissociation of N-cadherin adhesive bounds. Interestingly, the association of N-cadherin tail to catenins further regulates this exchange regime.

More stringent control over the adhesion ligand presentation, density, topology, rigidity and mobility is now necessary for further elucidation of the physical regulation of cadherin functions in cell-cell adhesion and associated responses.

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