FOCAL ADHESION-ASSOCIATED PROTEIN DYNAMICS ARE DETERMINED BY EXTRACELLULAR-MATRIX CONCENTRATION

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Cells are attached to different type of extracellular matrix and move by coupling forces generated in the actin cytoskeleton to the extracellular matrix via trans-membrane focal adhesions. Contraction of actin cytoskeleton linked to adhesion induces integrin clustering and focal adhesion maturation. The formation of focal adhesions is controlled by a mechanochemical mechanism in which cytoskeletal tensional forces drive changes in molecular assembly. Since the integrins are mechanotransducers that regulate cell fate, we hypothesized that matrix density and tension-induced maturation of focal adhesions result in changes in protein composition and dynamics that may influence adhesion morphology, strength and signalling. We first aimed to analyse the dynamics of different focal adhesion-associated proteins such as FAK and paxillin in control situation. Here, we describe a method to measure the binding rate and the mobile fraction of individual GFP-tagged focal adhesion-associated proteins by using FLIP (Fluorescence Loss In Photobleaching) - FRAP (Fluorescence Recovery After Photobleaching) technique and combining it with computer modelling [1]. With this method, we found that paxillin and FAK, binding partners share a similar diffusion coefficient (5\(\mu\)m\(^2\)/s) and a similar binding fraction (70\%). Surprisingly, both proteins which are well known binding partners, differ in their residence time at focal adhesions by a factor of 10 (10 s for FAK and 100 s for paxillin). Next, we determined the relationship between matrix concentration and protein binding/dissociation at focal adhesions. With the FLIP-FRAP method, we show that decreasing cellular traction forces on focal adhesions by low collagen matrices, increases the turnover of the focal adhesion protein FAK, and paxillin. These findings demonstrate that the molecular binding kinetics of some focal adhesion proteins are sensitive to mechanical environment.

Figure 1: example of FLIP-FRAP experiment on GFP-paxillin expressing cells (A) and analysis (B)