87 The dual role of Actin foci in Adhesion and Endocytosis of *Dictyostelium discoideum*

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The single-celled amoeba *Dictyostelium discoideum* (D.d.) is able to generally adhere to interfaces of different composition while hunting for bacteria in the soil. Up to now the process of adhesion and chemotactic migration has been subject to a manifold of studies [1] relying on several interface-sensitive methods [2, 3] but still not completely understood. D.d. does not express integrin containing focal adhesions [4], but instead presents actin-rich areas, so called actin foci, at the ventral membrane side [5], which are involved in force transmission, adhesion and migration [6]. However, several adhesion promoters of D.d. were identified while screening for endocytosis mutants. [7, 8] Here, we perform co-localization studies with fluorescence-tagged proteins for the dynamics of actin (mRFP reporter for LimE) and endocytosis (GFP tagged protein clathrin).

Before the experiment, axenic D.d. wildtype strains are cultured on petri dishes with HL5 medium (ForMedium™, UK) at 22 °C, washed and resuspended at $2.5*10^5$ cells in 1 ml phosphate buffer (PB, Sörensen). Upon experiment, cells are transferred to glass bottom dishes (µ-dish, 35mm, Ibidi, Germany) in medium-free PB and undergo a development to chemotactic competence and multicellularity (after 6-12h), which also reduces adhesion. Therefore cells were only analyzed in an interval of zero to three hours to guarantee comparability and no differentiation effects. The protein dynamics were assessed using Total Internal Reflection Fluorescence Microscopy (TIRFM) in conjunction with contact-area sensitive Reflection Interference Contrast Microscopy (RICM).

We focus on single actin foci appearing at the ventral side of the cell (Figure 1A{1-3}). Cell contour (yellow line, Figure 1A{1}) and single foci within the cell boundary (white line, Figure 1A{1}) were tracked by a custom Matlab script; exemplary temporal evolution of mRFP and GFP intensities as well as single actin foci RICM time series are shown in Figure 1B and C. As shown in Figure 1C we analyzed the time shift between the two TIRF signals for half-maximal width (red dot, green triangle) and the corresponding half-minimal width of the RICM intensity decrease (black square). Figure 1D left shows the averaged time difference (dt) of the appearing clathrin signal in comparison to the appearing actin signal (red line in the graph), while Figure 1D right shows the averaged time difference (dt) of the

RICM intensity drop to the appearing actin: Clathrin appears ~20 s before actin, while the individual contact area of the actin spot appears a bit earlier (~25s) than the clathrin signal. Since the intensity drop in RICM relates to reduce membrane-substrate distance, we can thus say that membrane dynamics precede the endocytosis and cytoskeletal events.

Generally we can say that – after endocytosis initiation monitored in RICM mode - a clathrin signal appears before the actin signal, hinting at the budding of the clathrin-mediated endocytotic vesicle upon delayed actin activity. First analysis of the spatial coincidence of the two TIRF signals however shows that only 68 % (N=1442) of the actin foci appear on a clathrin location, while the rest has no co-localization with clathrin signals over imaging intervals of several minutes, thus underlining the role of actin foci both and independently in adhesion and endocytosis.

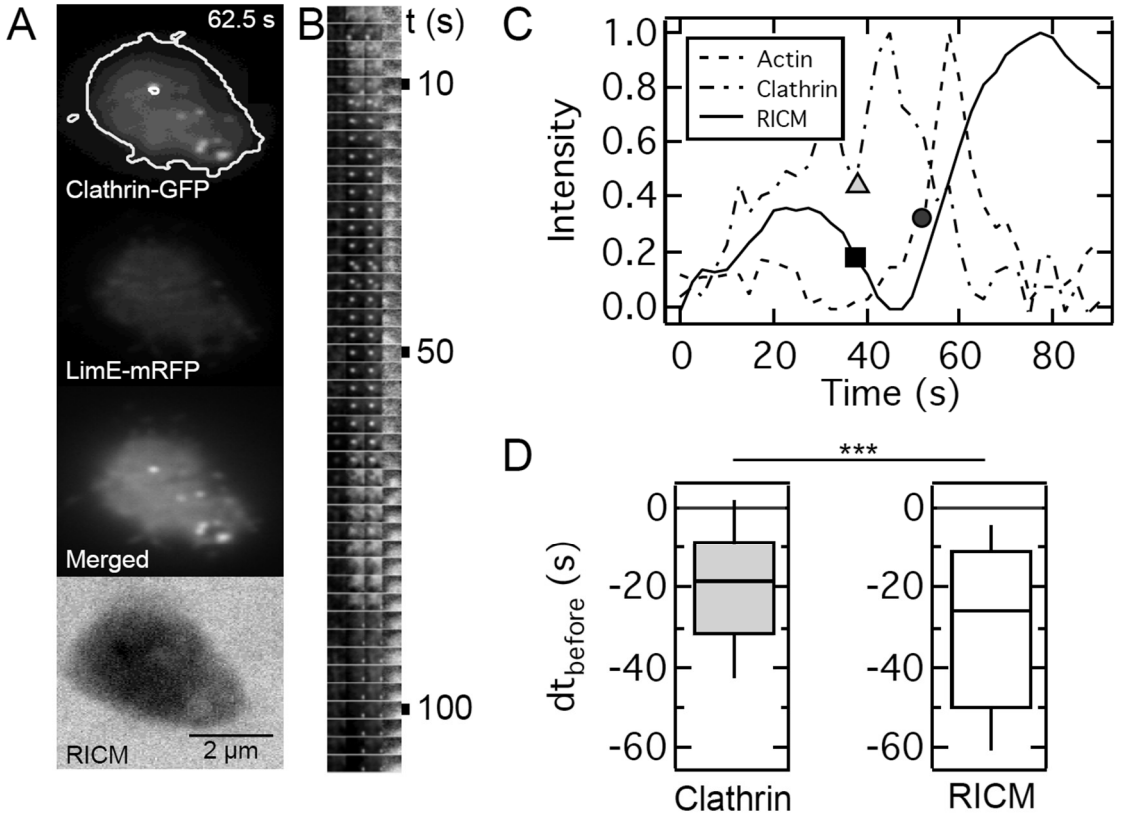

Fig. 1: **A:** TIRF-micrograph of a single D.d. cell (from top to bottom: Endocytotic vesicles labelled via Clathrin-GFP, actin labeled with LimE-mRFP, merged TIRF image, RICM based contact area). **B:** Time series of exemplary focus highlighted in A (categories/colors correspond to A). **C:** Intensity profile of labeled focus (white, A) and reference time points. **D:** Analysis of the clathrin and RICM time shift (half-width) of the intensity before the signal reaches it's maximum in reference to the actin signal (red line). Clathrin appears ~20 s before the actin signal appears. The membrane-substrate distance changed significantly, with the membrane moving closer to the substrate (~25 s).

87.1 References

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