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Correlating pathogen behavior with host responses during pathogen invasion on the single cell level via innovative fluorescence microscopy

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One strategy employed by a number of pathogens is the secretion of bacterial effector proteins into host cells via sophisticated secretion machineries (1,2). The type III secretion system is one of such machines resembling a molecular syringe. A particularity of the type III secretion needles is their capacity to translocate bacterial effectors in a single step across three membrane layers- the inner and outer bacteria membranes, and the host plasma membrane. The entero-invasive pathogen *Shigella* uses this molecular needle to inject about 25 bacterial effectors into the host cells leading to the uptake of the bacterium by typically non-phagocytic host cells, and furthermore the injected undermine the host immune system. We have set up a novel methodology for direct fluorescent labelling of bacterial effectors without functional loss within living bacteria (1). Using rapid multidimensional fluorescence microscopy, we can track the pace of effector secretion from individual bacteria (see figure 1). This enables the comparison of the secretion kinetics of the secreted effectors. The next step of *Shigella* invasion is the rupture of the endocytic vacuole after uptake. Generally, cellular uptake of bacterial pathogens leads to (i) blocking the progression of the bacteria-containing endosomal vacuoles and their subsequent fusion with lysosomes (e.g. *M. tuberculosis*), (ii) altering the composition of the endosomal vacuoles that appear advantageous to the invading pathogen (e.g. *Salmonella*), or (iii) escaping the endocytic vacuole via rupture of the enclosing membrane (e.g. *Shigella*). Studying these events has given novel insight into the mode of function of the participating constituents of the vesicular trafficking machine. However, the overall sequence and hierarchy of the involved steps are still poorly understood due to the limited number of approaches that allow dynamic and functional investigations on the single cell level. We have established novel fluorescence microscopy based approaches that give precise information on the intracellular localization of bacterial pathogens with very high temporal resolution (figure 2). With our novel assays, we are able to study how bacterial factors communicate with host factors to shape the localization of the pathogens during cellular uptake. During the internalization of bacterial pathogens, a number of immune genes is induced. The expression of host genes depends on the behavior of the pathogens and on their intracellular localization. Common reporters for gene expression, like GFP or luciferase, have the disadvantage that they require to maturation- a process that is taking time and its length is difficult to predict (3). Furthermore, other approaches for gene profiling, like gene chips require the disruption of the cells and collecting transcripts from a large number of cells. To overcome these problems we have developed novel reporter systems that allow gene profiling of specific immune genes in single living cells. Overall, we are able to combine our novel approaches with assays that track the localization of invading bacteria and their physiological behavior. This leads to integrative information on the invasion strategies employed by the pathogens and on the induced host responses.

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[1] J. Enninga et al. Nature Methods 2 (2005) 959
[2] J. Enninga et al. Trends Microbiol. 11 (2007) 483
[3] G. Cabal, J. Enninga, MM. Mhlanga. *Single Molecule Tracking inside Living Samples.* Springer Publishers. 2007
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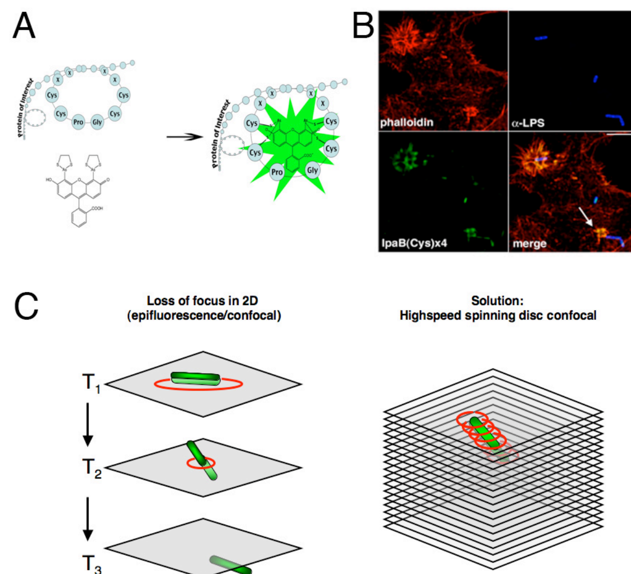

FIG. 1. **A)** Schematics of the 4Cys-FlAsH labelling approach for fluorescently labelling type III effectors inside bacteria without functional loss. The metallo-organic FlAsH compound forms a high-affinity complex with 4Cys sequences that can be added to the effectors. **B)** 4Cys-tagged and FlAsH labelled effectors are secreted from invading Shigella (green). The bacteria have been labelled with anti-LPS (blue), and the actin foci are highlighted by phalloidin-rhodamine (red). **C)** Set-up for tracking the secretion of fluorescent effectors (via the 4Cys-FlAsH labelling approach) from single bacteria. During the time-course of the experiment, a thick volume has to be observed because the events are taking place in three dimensions. Then, the fluorescence intensities can be integrated from single bacteria and they can be correlated with the remaining pool of fluorescent effectors within the bacteria.

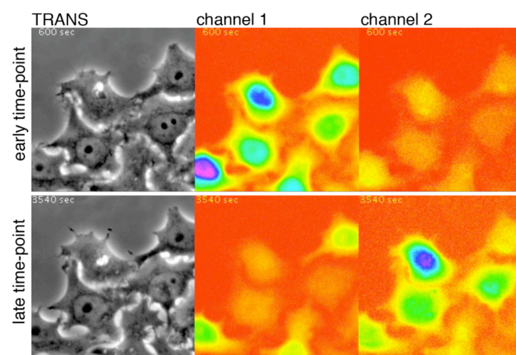

FIG. 2. During the invasion of host cells, *Shigella* escape into the host cytoplasm. The Trans channel illustrates the invading bacteria. With a novel fluorescent microscopic approach, we can follow the intracellular localization of the invading bacteria. Before the escape from the vacuole, the host cells fluoresce in one channel, after the escape, the cells fluoresce in another fluorescent channel.

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